PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 31/46, C07D 489/12
G01N 33/567

A1

(11) International Publication Number: WO 94/06426

(43) International Publication Date: 31 March 1994 (31.03.94)

(21) International Application Number: PCT/US93/08869

(22) International Filing Date: 17 September 1993 (17.09.93)

(30) Priority data:

| | | |
|---|---|---|
| 947,690 | 21 September 1992 (21.09.92) | US |
| 977,322 | 17 November 1992 (17.11.92) | US |
| 088,503 | 7 July 1993 (07.07.93) | US |

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*

(54) Title: METHODS FOR IDENTIFYING AND USING LOW/NON-ADDICTIVE OPIOID ANALGESICS

(57) Abstract

The present invention relates to a method of using a bioassay consisting of an electrophysiological method and a cell culture system of dorsal-root ganglion (DRG) neurons to screen and identify opioids with a high potential for use as "low- or non-addictive" analgesics. Another aspect of the invention relates to a specific group of opioid alkaloids and analogues thereof identified by the bioassay of the invention for the unique ability to activate only inhibitory, but not excitatory, opioid receptor function, for use as low- or non-addictive analgesics. Another aspect of the invention relates to the specific use of etorphine or dihydroetorphine of the opioid alkaloid family as low- or non-addictive analgesics and for the treatment of opioid addiction. The present invention also relates to the preparation of dihydroetorphine hydrochloride (7α-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethanotetrahydrooripavine hydrochloride) and a pharmaceutical composition comprising the compound as an active ingredient in the form of a pharmaceutically acceptable salt.

# METHODS FOR IDENTIFYING AND USING LOW/NON-ADDICTIVE OPIOID ANALGESICS

The present invention relates to a specific group of opioid agonists for use as low/non-addictive analgesics and for treatment of opioid addiction. More particularly, the present invention is directed to etorphine, dihydroetorphine, ohmefentanyl and other opioid and analogues thereof that are effective as low/non-addictive analgesics and for the treatment of opioid addiction. In addition, this invention provides a bioassay method to screen and identify such compounds with the ability to selectively activate inhibitory but not excitatory opioid receptor-mediated functions.

The present invention also relates to the preparation of etorphine, dihydroetorphine and analogues thereof using thebaine as the starting material. More specifically, the present invention relates to the preparation of dihydroetorphine hydrochloride (7α-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethano-tetrahydrooripavine hydrochloride) and pharmaceutical compositions thereof.

Since the introduction of morphine [Fig. 1(I)] to the clinic as a pain reliever, clinicians have been troubled with the problem of drug addiction. For more than a century, chemists, pharmacologists, and clinicians have strived to find an ideal analgesic with high potency, yet low addictivity. A series of opioids such as meperidine, methadone [Fig. 1(II)], and fentanyl were subsequently developed.

However, none of these drugs exert sustained analgesic effects in patients without developing addiction. In Western countries, methadone substitution has been employed for the treatment of drug abuse for some time. Unfortunately, methadone induces significant psychological and physical dependencies. Consequently, patients

undergoing such treatment usually convert to methadone dependence during withdrawal from chronic use of morphine, heroin or other opioids (Jaffe, 1990). Therefore the need remains to develop better methods based upon insights into the molecular and cellular mechanisms underlying opioid addiction for treating drug abuse and particularly a means to identify compounds for use as low- or non-addictive analgesics and for suppression of opioid withdrawal symptoms.

The present invention is directed to an in vitro screening method for identifying a low- or non-addictive opioid analgesic by screening opioids to identify a compound which is capable of evoking an inhibitory effect but not an excitatory effect on opioid receptor-mediated functions of sensory neurons in a dose-dependent manner over the concentration range of from about femtomolar (fM) to about micromolar (μM). In particular, such opioid compounds are identified by recording the action potential duration (APD) of a sensory neuron elicited by the compound in a cell culture screening assay and selecting those opioid compounds which shorten the APD but do not prolong the APD relative to a control APD when the compounds are assayed in the concentration range of about fM to about μM. Opioid compounds with these characteristics are thereby identified as low- or non-addictive opioid analgesics of the invention. Preferably, the cell culture screening assay comprises exposing a dorsal-root ganglion (DRG) neuron to the candidate compound, typically by bath perfusion, applying a brief intracellular depolarizing current to said DRG neuron, and recording opioid-induced alteration in the APD of the DRG neuron using standard electrophysiological techniques.

Another aspect of the invention, thus, provides low- or non-addictive analgesics, particularly as identified by the method of the present invention, which are capable of evoking the inhibitory but not the excitatory effects of opioid receptor-mediated functions, particularly on sensory

neurons, in a dose-dependent manner in concentrations ranging from about fM to about μM. In a preferred embodiment these opioids include etorphine, dihydroetorphine or ohmefentanyl. Pharmaceutical compositions containing the subject low- or non-addictive opioids, or pharmaceutically acceptable salts thereof, together with pharmaceutically acceptable carriers are also provided. In addition, the subject pharmaceutical compositions can also contain a replacement opioid or naloxone.

Yet another aspect of this invention provides a method of treating opioid addiction by administering an effective amount of a non-addictive opioid analgesic, or an analog thereof, to a patient for a time sufficient to relieve or suppress withdrawal symptoms that occur when the addictive opioid is withheld from the addict. After the initial administration of the non-addictive opioid analgesic for a period to permit alleviation of the withdrawal symptoms, the dose of the non-addictive opioid analgesic is gradually decreased from the original dose to zero over a time sufficient to fully wean said patient from said analgesic without untoward side effects. Typically the initial administration of the non-addictive opioid analgesic lasts for about 1 to about 5 days and the weaning period lasts from about 1 to about 7 days, so that a patient can be withdrawn from opioid addiction within an overall about 2 to 12 day period. In a preferred embodiment the non-addictive opioid analgesic is etorphine or dihydroetorphine initially administered at a dose of from about 10 μg to about 1000 μg per day. Such dosages are usually administered sublingually, intramuscularly or intravenously, preferably by intravenous dripping, depending on the severity of the withdrawal symptoms in the patient. Even more preferably, opioid addiction is treated by administering about 40 to about 500 μg of dihydroetorphine per day to a patient for about one to about three days, administering a decreasing amount of dihydroetorphine for the following about four to

about seven days so that no further dihydroetorphine is necessary by about 10 days after the first administration of dihydroetorphine.

A further aspect of the invention provides a method of treating opioid addiction by administering an effective amount of a non-addictive opioid analgesic, to a patient for a time sufficient for immediate relief or suppression of withdrawal symptoms due to said opioid addiction; administering an effective amount of a longer-acting replacement opioid for a time sufficient to maintain the relief or suppression of withdrawal symptoms, followed by administering a decreasing dose of the non-addictive opioid analgesic for a time sufficient to wean said patient from said opioid analgesic without untoward side effects. Typically the initial administration of the non-addictive opioid analgesic lasts for about 1 to about 3 days, the administration of the replacement opioid lasts for about 1 to about 3 days, and the return to the non-addictive opioid analgesic with its concomitant weaning period lasts from about 1 to about 8 days, so that a patient can be withdrawn from opioid addiction within an overall 3 to 14 day period. Alternatively, the initial administration of the non-addictive opioid analgesics and administration of the replacement opioid can be made simultaneously. Thus, these two opioids are co-administered until the patient is relieved of withdrawal symptoms (e.g. the symptoms are effectively suppressed). Thereafter, administration of the replacement opioid is discontinued and the non-addictive opioid analgesic dosage is stepwise or gradually reduced until the patient is weaned off of the non-addictive opioid analgesic. The time periods for co-administering these opioids is about 2 to about 6 days and for weaning is about 1 to about 8 days, so that the patient can be withdrawn from opioid addiction within an about 3 to about 14 day period. In a preferred embodiment the non-addictive opioid analgesic is etorphine or dihydroetorphine initially administered at a

dose of from about 10 μg to about 1000 μg per day. Such dosages are usually administered sublingually, intramuscularly or by intravenous dripping depending on the severity of the withdrawal symptoms in the patient. Preferably the replacement opioid is methadone administered per os at a dose of about 5-100 mg/day.

A still further aspect of the invention provides a method of treating acute or chronic pain with a low- or non-addictive opioid analgesic. In particular, dihydroetorphine hydrochloride (DHE) is administered to a patient for a time and in an amount effective to relieve or suppress pain without resultant addiction. Treatment for acute pain is typically accomplished by administration of about 20-60 μg DHE sublingually, up to about 180 μg per day for the duration of the pain, and typically no longer than 1 week. Treatment for chronic pain is typically accomplished by administration of about 20-100 μg DHE sublingually, up to 400 μg per day, and such administration can last several months. In rare instances treatment of chronic pain can result in mild addiction.

Alternatively, chronic or acute pain can be treated by co-administering a low- or non-addictive opioid analgesic and a replacement opioid for a time and in an amount effective to relieve or suppress pain without resultant addiction. The potent inhibitory effects exerted by low- or non-addictive opioid analgesics, such as DHE and etorphine, block the excitatory effects exerted by replacement opioids such as morphine and methadone. Typically the amounts of the non-addictive opioid analgesics are about 10 to about 1000 μg per day as well as those dosages described above for treatment of pain with only a low- or non-addictive opioid analgesic. The amounts of the replacement opioid are about 5 to about 100 mg per day. Dosages of the analgesic can also be determined as about 0.05% to about 5% of the replacement opioid on a weight basis. Using a combination of replacement opioid with a relatively lesser amount of a

low- or non-addictive opioid analgesic permits treatment of pain without addiction or with a low incidence of addiction. Preferably the analgesic is DHE, etorphine, ohmefentanyl or a pharmaceutically acceptable salt thereof, and the replacement opioid is morphine, methadone or fentanyl.

The present invention also relates to improved methods for the preparation of etorphine, DHE, and analogues thereof using thebaine as the starting material. For example, the present invention provides a method for the preparation of dihydroetorphine hydrochloride (7α-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethano-tetrahydrooripavine hydrochloride) and other salts of DHE.

In particular, the method for preparing dihydroetorphine and its analogues [Figs. 13, 14 and 15] comprises (1) reacting thebaine with an excess of methyl vinyl ketone for a time and under conditions sufficient to produce a first product and recovering that first product; subjecting the first product to catalytic hydrogenation to produce a second product and recovering that second product; reacting the second product with a Grignard reagent of the formula RMgX for a time and under conditions to produce a third product and recovering that third product; reacting the third product with a strong base in an anhydrous solution for a time and under conditions sufficient to produce dihydroetorphine or its corresponding analog, wherein R is a lower alkyl group and X is a halogen. The R group is preferably n-propyl or i-amyl. The method of preparing etorphine and its analogues in accordance with this invention follows the same method for preparation of DHE except that catalytic hydrogenation step is omitted. When preparing etorphine or etorphine-related components, R is preferably n-propyl, n-butyl, n-amyl, i-amyl or cyclohexyl.

Fig. 1 depicts the structure of opioids: (I) morphine; (II) methadone; (III) etorphine (a) and analogues (b,c,d,e) thereof, (IV) dihydroetorphine(a) and an analogue (b)

thereof, and (V) naloxone.

Fig. 2 illustrates positive- and negative-feedback phosphorylation mechanisms in dorsal root ganglion (DRG) neurons that may result in opioid excitatory supersensitivity and opioid inhibitory desensitization during chronic opioid exposure. Sustained activation of excitatory Gs-coupled opioid receptors increases adenylate cyclase activities and PKA, resulting in: (a) cAMP-dependent elevation of GM1 ganglioside via PKA, and (b) activation of voltage-sensitive $K^+$ and $Ca^{2+}$ channels, leading to action potential duration (APD) prolongation and enhanced transmitter release (if similar APD modulation occurs in presynaptic DRG terminals). Elevation of GM1, in turn, enhances the efficacy of excitatory Gs-coupled opioid receptor functions, i.e., heterologous sensitization (resulting in dependence). The upregulated AC/cAMP/PKA system may concomitantly phosphorylate ligand-bound inhibitory opioid receptors, thereby attenuating their coupling to Gi/Go, i.e., heterologous desensitization (resulting in tolerance to opioid inhibitory effects). Abbreviations: AC, adenylate cyclase; PKA, cAMP-dependent protein kinase; $g_K$, membrane $K^+$ conductance; $g_{Ca}$, membrane $Ca^{2+}$ conductance.

Fig. 3 illustrates that acute application of pM-$\mu$M concentrations of etorphine to a naive DRG neuron elicits inhibitory shortening of the APD. 1: Action potential (AP) generated by a DRG neuron in Hank's balanced salt solution containing 5 mM $Ca^{2+}$ and 5 mM $Ba^{2+}$ (BSS). AP response in this record (and in all records below) was evoked by a brief (2 msec) intracellular depolarizing current pulse. 2-5: The APD is progressively shortened by bath perfusion of 1 fM, 1 pM, 1 nM and 1 $\mu$M etorphine, respectively. 6: After washout of etorphine, the APD recovers.

Fig. 4 shows the dose-response relationship of etorphine, DHE and dynorphin (1-13) (Dyn 1-13) effects on the APD of DRG neurons. Etorphine and DHE elicited a dose-

dependent shortening of the APD (n=11 and 13, respectively). In contrast, Dyn (1-13) elicited a dose-dependent prolongation of the APD at fM-nM concentrations and required much higher concentrations (ca. μM) to shorten the APD (n=35).

Fig. 5 illustrates that chronic exposure of a DRG neuron to a bimodally acting opioid (DADLE) causes the DRG neuron to become supersensitive to the excitatory effects of dynorphin (1-13) (Dyn), whereas perfusion of etorphine effectively shortened the APD of the same DRG neuron (inhibitory response). 1: Action potential generated by a DRG neuron treated for 3 wks in culture with 1 μM DADLE and then tested in BSS with 1 μM DADLE. 2: APD is prolonged by bath perfusion of 1 fM Dyn with 1 μM DADLE (5 min test). 3,4: APD is further prolonged by sequentially raising the Dyn concentration to 1 nM and 1 μM (5 min tests). 5: Control response 5 min after washout of Dyn with BSS containing 1 μM DADLE. 6: 1 fM etorphine (Etorp) shortens the APD of the same DRG neuron in the presence of 1 μM DADLE. 7-9: Further increases in the concentration of etorphine from 1 pM to 1 μM progressively shorten the APD. 10: APD returns to control value after removal of etorphine.

Fig. 6 shows that chronic exposure to a bimodally acting opioid (DADLE) followed by acute application of low concentrations of etorphine can block the excitatory APD-prolonging effects precipitated by naloxone (NLX) in this supersensitive DRG neurons. 1: Action potential generated by a DRG neuron treated for 2 wks in culture with 1 μM DADLE and then tested in BSS with 1 μM DADLE. 2: 1 nM NLX prolongs the APD of this DRG neuron (5 min test). In contrast, nM naloxone is ineffective on naive DRG neurons (Crain & Shen, 1992a,b) 3: Acute addition of 1 pM etorphine attenuates the naloxone-induced APD prolongation (5 min test). 4: Further increase in concentration of etorphine to 1 nM almost completely blocks the naloxone-induced APD prolongation.

Fig. 7 illustrates the relief of naloxone-precipitated, sustained body weight loss by morphine, DHE and methadone injections in morphine-dependent rats. Daily dose: morphine 100 mg/kg, divided into 2 subdoses; DHE 12 μg/kg, divided into 4 subdoses; methadone 24 mg/kg, divided into 4 subdoses. Filled circle: morphine group; open circle: DHE group; filled triangle: methadone; cross: saline control group. X ± SD, ***$p<0.01$, as compared with saline control group.

Fig. 8 depicts the effect of DHE and methadone substitution on naloxone precipitated body weight loss in morphine-dependent rats. The body weight loss from the first naloxone precipitation test is provided in Column A. The second naloxone precipitation test was performed after 4 days of maintaining one group of rats with morphine (100 mg/kg/day, divided into 2 subdoses), a second group with DHE (12 μg/kg/day, divided into 4 subdoses) and a third group with methadone (24 mg/day, divided into 4 subdoses). The body weight loss after the second naloxone precipitation test is provided in Column B. Statistical p values between the first and second naloxone precipitation test are "**", $p<0.05$ and "***", $p<0.01$. The p value for the DHE group relative to the methadone group is $p<0.05$.

Fig. 9 depicts the withdrawal symptom scores after naloxone precipitation for DHE and methadone substitution in morphine-dependent rats. Rats were treated as described in Fig. 8. Column A: Withdrawal scores from the first naloxone precipitation test. Column B: Withdrawal scores from the second naloxone precipitation test. Statistical p values between the first and second naloxone precipitation test are "**", $p<0.05$ and "***", $p<0.01$. The p value for the DHE group relative to the methadone group is $p<0.01$.

Fig. 10 shows the development of withdrawal symptoms in morphine-dependent monkey after cessation of morphine.

Fig. 11 illustrates the relief of withdrawal symptoms by DHE in morphine-dependent monkeys. The arrows indicate

time of DHE injection (3 μg/kg). Open circle: control group; filled circle: DHE group.

Fig. 12 illustrates the therapeutic effect of DHE and methadone on withdrawal symptoms of morphine-dependent monkeys. The arrows indicate the time of naloxone (NLX) precipitation (1 mg/kg). Open circle: control group; filled circle: DHE group; filled triangle: methadone group.

Figure 13 illustrates a reaction scheme for the synthesis of etorphine and analogues thereof from thebaine.

Figure 14 illustrates a reaction scheme for the synthesis of dihydroetorphine and analogues thereof from thebaine.

Figure 15 illustrates the synthesis of dihydroetorphine hydrochloride from thebaine.

## BRIEF DESCRIPTION OF ABBREVIATIONS USED

| | |
|---|---|
| DADLE | [D-Ala$^2$,D-Leu$^5$]enkephalin |
| DAGO | [D-Ala$^2$,MePhe$^4$,Gly-ol]enkephalin |
| DPDPE | Tyr-D-Pen-Gly-Phe-D-Pen (Pen = penicillamine) |
| U-50,488H | 3,4 dichloro-N-methyl-N-(2-[1-pyrrolidinyl]-cyclohexyl)benzene-acetamide |
| Dynorphin 1-13 | dynorphin A, Fragment 1-13 (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys) |
| Dynorphin 1-17 | dynorphin A, Fragment 1-17 (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln) |
| Etor | etorphine |
| DHE | dihydroetorphine |

## DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, electrophysiologic assay of the effects of opioids on the action potential duration of sensory neurons in organotypic cultures provides an extremely sensitive *in vitro* bioassay to screen and characterize agonists with the ability to activate inhibitory, but not excitatory, opioid receptor-mediated functions. This assay permits

identification of low- or non-addictive opioid analgesics as well as agents useful for treatment of drug addiction.

As used herein "non-addictive" and "low-addictive" are used interchangeably to describe the addiction potential of the opioids of the present invention. In treating opioid addiction in accordance with the present invention, the subject opioids are essentially non-addictive at the prescribed dosages when used for periods of up to several weeks. For example, DHE administration to a drug addict in the range of about 10 μg to about 1000 μg per day which is gradually withdrawn over a period of up to 14 days, does not result in addiction to DHE. In contrast, even well-controlled treatment of drug addicts by methadone substitution invariably results in transfer to methadone addiction. The present invention, thus, greatly improves the present methods for treating opiate drug abuse without concomitant addiction to another opioid.

Likewise, treatment of acute pain for several days with the subject opioids in accordance with the present invention, does not result in addiction. While treatment of chronic pain of long duration with the subject opioids generally does not result in addiction, mild addiction may result in exceptional cases. Hence use of the subject opioids in accordance with this invention is non-addictive for the vast majority of chronic pain patients. The addiction potential of the subject opioids, as illustrated with DHE, for chronic pain patients is thus low, typically less than 1 in 100 for patients treated longer than 3 months. For example, no addiction has been observed when treating patients for up to 3 months with DHE. Moreover, in the rare cases of addiction, such addiction may result from minor contaminants present in bimodally-acting thebaine, a starting material for drug synthesis which is carried into certain preparations of DHE.

As used herein "opioid" refers to any substance that binds specifically to an opiate receptor (Casy & Parfitt,

1986; Pasternak 1988).

As used herein "replacement opioid" is a bimodally-acting opioid that has both inhibitory and excitatory effects on opioid receptors. Such opioids, generally, have a longer duration of action than the non-addictive opioid analgesics with which it is combined for use. Combination therapy of replacement opioids with non-addictive opioid analgesics permit the latter compounds to block or mask the excitatory effects of the replacement opioids.

Activation of opioid receptors has been known to produce inhibitory effects on neuronal activity which in turn provides the primary cellular mechanism underlying opioid analgesia in vivo (e.g. North, 1986). However, recent electrophysiological studies indicated that specific mu-, delta- and kappa-opioid receptor agonists elicit both excitatory and inhibitory modulation of the action potentials of sensory DRG neurons isolated in culture in a concentration dependent manner (Shen & Crain, 1989; Crain & Shen, 1990).

These opioid agonists were found to elicit excitatory effects at low (<nM) concentrations and inhibitory effects at high ($\mu$M) concentrations as measured by prolongation or shortening of the calcium-dependent component of the action potential duration (APD), respectively (Fig. 2; Table 1).

Earlier experiments demonstrated that the excitatory effects of opioids are mediated by opioid receptors that are positively coupled via a cholera toxin-sensitive Gs-like regulatory protein to adenyl cyclase and cyclic AMP-dependent voltage-sensitive ionic conductances (resembling, for example, beta-adrenergic receptors)(Fig. 2; Shen & Crain, 1989, 1990a; Crain & Shen, 1990, 1992), whereas inhibitory effects are mediated by opioid receptors linked to pertussis toxin-sensitive Gi/Go proteins (resembling alpha$_2$-adrenergic receptors)(Fig. 2; Shen & Crain, 1989; Gross et al, 1990).

The ability to differentiate between these bimodal

properties of opioids, i.e. excitatory and inhibitory activities mediated by two distinct groups of opioid receptors, has led to the present invention, and particularly to a method for identifying low- or non-addictive opioid analgesics. Hence, this method provides an in vitro bioassay to identify compounds that can selectively activate the inhibitory but not excitatory opioid response. Since sustained activation of excitatory opioid receptor functions plays a crucial role in development of tolerance and dependence in chronic opioid-treated neurons in vitro (Crain & Shen, 1992; Shen & Crain, 1992), compounds with such properties, i.e. which activate the inhibitory response but not the excitatory response, are useful as non-addictive analgesics in vivo.

In particular, the in vitro bioassay uses a cell culture system of DRG neurons to screen candidate compounds by exposing the DRG neurons to the candidate compound and observing its effect on the APD using standard electrophysiological recording methods. The detailed methodology for growing neurons, treating with a candidate compound and recording the APD are provided in Example 1. Any opioid compound screened by this bioassay that exhibits inhibitory effects (e.g., shortening the APD in DRG neurons) but not excitatory effects (e.g., prolonging the APD in DRG neurons) in about the fM-pM range to $\mu$M range is a low- or non-addictive opioid analgesic in vivo. Generally, these compounds effect the APD in a concentration-dependent manner and the responses are mediated by specific opioid receptors. Hence, the method of the present invention provides a powerful tool to identify low- or non-addictive opioid analgesics.

Nearly all the opioids tested by this bioassay, including morphine, enkephalins, dynorphins, endorphins and synthetic opioid peptides, have dose-related dual modulatory effects (i.e. both inhibitory and excitatory) on the action potential of sensory DRG neurons. All such compounds are

well-known to be addictive. However, in accordance with this invention etorphine and dihydroetorphine (thebaine derivatives) (Bentley and Hardy, 1963; Bentley and Hardy, 1967), compounds previously believed and classified as addictive (WHO Rep 1966), have the selective characteristic (Table 1) of inhibiting opioid-receptor mediated functions without exciting such functions. Both etorphine and dihydroetorphine elicit dose-dependent (inhibitory) shortening of the APD, starting at about pM levels in some of the DRG neurons, and reaching a maximum effect at μM levels in most of the DRG neurons (Example 1). Furthermore, no excitatory prolongation of the APD occurs with these two compounds at <pM concentrations in contrast to the characteristic excitatory effects elicited at low concentration by the bimodally-acting opioids.

It is well known that chronic exposure of DRG-spinal cord explants to bimodally-acting opioids (e.g., morphine or DADLE) causes sensory DRG neurons to become desensitized to the inhibitory effects of opioid agonists, resulting in tolerance (Crain et al, 1988), and supersensitized to the excitatory effects of opioid agonists as well as antagonists, resembling significant features of abstinence, dependence and withdrawal syndrome in vivo (Crain & Shen, 1992a,b; Shen & Crain, 1992).

Sustained activation of excitatory opioid receptors after chronic treatment with an opioid agonist triggers a positive-feedback mechanism that results in up-regulation of a Gs/adenylate cyclase/cyclic AMP/protein kinase A/GM1 glycosyl-transferase system that may account for the remarkable supersensitivity of chronically opioid-treated neurons to the excitatory effects of opioid antagonists and agonists (Fig. 2, Crain & Shen, 1992a,b; Shen & Crain, 1992).

When DRG-cord explants are chronically treated with a bimodally-acting delta/mu agonist, DADLE (1 μM) or morphine (1 μg/ml) for 3 weeks, acute treatment with etorphine still

elicits a marked inhibitory dose-dependent shortening of the APD of DRG neurons even at concentrations as low as 1 fM (Example 2), whereas bimodally-acting mu, delta and kappa opioid agonists show a high degree of opioid excitatory supersensitivity at concentrations ranging from pM to μM (Example 2).

Furthermore, the excitatory APD prolongation of chronic opioid-treated DRG neurons precipitated by acute application of nM naloxone [Fig. 1(V)](Crain & Shen, 1992a,b), which provides a cellular model of naloxone-induced withdrawal supersensitivity in opiate addicts in vivo (Crain & Shen, 1992b), can be blocked by acute application of etorphine, but not by morphine or other bimodally acting opioid agonists (Example 2).

Tissue culture studies provide strong support that excitatory opioid receptor functions of sensory neurons play important roles in vivo, both by attenuating analgesic effects mediated by inhibitory opioid receptors and by facilitating the cellular mechanisms underlying addiction. The use of opioids (e.g. etorphine, dihydroetorphine), that at low concentrations preferentially activate inhibitory but not excitatory opioid receptor functions in vitro, as indicated by the screening model, results in much more potent analgesia in vivo and far less evidence of dependence/addiction than occurs during chronic treatment with morphine and most other bimodally-acting opioids.

The present invention demonstrates that etorphine (and compounds with similar properties as identified by the present bioassay (e.g. dihydroetorphine and ohmefentanyl) elicits potent dose-dependent inhibitory APD-shortening effects on naive and chronic opioid-treated, "addicted" sensory DRG neurons, even at low (pM-nM) concentrations where most bimodally-acting opioids generally elicit excitatory APD-prolonging effects. Hence etorphine and similar compounds of this invention selectively activate inhibitory rather than excitatory opioid receptors on DRG

neurons, even when the cells are supersensitive to the excitatory effects of bimodally-acting opioids following chronic treatment.

Etorphine has long been known to be >1,000 times more potent than morphine as an analgesic in animals (Blane et al, 1967) and humans (Blane & Robbie, 1970; Jasinski et al, 1975). This invention shows that the high inhibitory potency of etorphine may be due, in part, to its selective activation of inhibitory opioid receptors whose effects are not attenuated by the concomitant activation of higher-affinity excitatory opioid receptors.

The clinical trial results of the present invention show that low doses of dihydroetorphine, a specifically inhibitory opioid-receptor agonist, are remarkably effective in relieving postoperative pain and chronic pain in terminal cancer patients, yet tolerance and addiction are far less evident than observed with morphine and other conventional bimodally-acting opioids (Example 5). Thousands of patients have been treated with a >90% effective rate and no significant adverse side-effects have been observed. Furthermore, the potent inhibitory effect exerted by the low- or non-addictive opioid analgesics of this invention can block or suppress the excitatory effects of bimodally-acting opioids, i.e. the replacement opioid as defined herein, to alleviate tolerance and addiction commonly observed by sole usage of bimodally-acting opioids (e.g. morphine or methadone).

In addition, several hundred heroin addicts have been successfully treated over a two year period. In this group, withdrawal symptoms were rapidly blocked and dihydroetorphine substitution therapy was maintained for about a week with minimal rebound after final opioid withdrawal (Example 6). Similar results were obtained in tests on morphine-dependent monkeys and rats (Examples 3 & 4). The successful results obtained with dihydroetorphine in treating heroin and morphine addiction are in sharp

contrast to the unreliable results obtained in comparative clinical studies with methadone and other bimodally-acting or mixed agonist-antagonist opioids.

Hence, another aspect of the present invention provides a method of treating opioid addiction by administering an opioid or analog therof, in an amount effective and for a time sufficient to relieve the withdrawal symptoms of opioid addiction and subsequently withdrawing administration of said opioid or analog thereof.

Another aspect of the present invention provides improved synthetic methods for the preparation of DHE, etorphine and analogs of these compounds. In addition, a method for preparing salts, particularly pharmaceutically acceptable salts, of the foregoing compounds is also provided.

The reaction scheme for preparing etorphine (Fig. 1 (III)) and related analogues is shown in Fig. 13. As depicted, thebaine [1] is reacted with an excess of methyl vinyl ketone under reflux for about 1 hour. Any remaining ketone is then distilled off, preferably under pressure. The thick oil can be dissolved in warm methanol, cooled to allow crystallization and the crystals recovered. Conveniently, the crystals can be washed several times with ice cold methanol and dried to yield 7"-acetyl-6,14-endo-etheno-tetrahydrothebaine [2]. This compound [2] is then reacted with a Grignard reagent of the formula RMgX to form a tertiary alcohol with the R group of the Grignard reagent at the 7" position of thebaine as represented by compound [3] in Fig. 13.

The R group of the Grignard reagent is lower alkyl and X is a halogen. Analogs of etorphine are thus prepared by varying the R group. As used herein lower alkyl refers to alkyl groups containing one to six carbon atoms. These groups may be straight, branched or cyclic chains and include such groups as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, t-butyl, pentyl (amyl),

isopentyl (i-amyl), neopentyl (n-amyl), hexyl, cyclopentyl, cyclohexyl and the like. When R is propyl, the final product [4] is etorphine. Preferred R groups for etorphine and its analogues are n-propyl, n-butyl, n-amyl, i-amyl or cyclohexyl.

Compound [3] is thus prepared by reacting an anhydrous solution of [2] with the desired Grignard reagent for a time and under conditions for the formation of the tertiary alcohol. For example, [2] can be dissolved in benzene and refluxed with the Grignard reagent for several hours, or as needed, until the reaction goes to completion. Upon completion, the anhydrous solution can be added to an immiscible aqueous solution (e.g., saturated ammonium chloride), the product extracted into that aqueous solution, followed by separation of the organic and aqueous layers. The aqueous layer is recovered and extracted with ether or other suitable solvent several times to yield a neutral solution containing compound [3]. Compound [3] can be further purified by recrystallization.

Reaction of compound [3] under strong basic conditions yields the 7°-[1-hydroxy-1-methylR]-6,14-endo-etheno-tetrahydrooripavine compounds [4]. Such compounds [4] can be recovered by extraction, filtration, recrystallization and the like. When R is n-propyl, then [4] is etorphine.

Any of various salts of etorphine or its analogues [4] can be prepared by reacting the free base with the desired free acid and recovering the resultant salt by crystallization, filtration or the like. In a preferred embodiment, [4] is dissolved in an alcoholic ether solution and an ether solution containing the desired acid is added thereto until the reaction mixture reaches a pH of about 2. Additional ether is added thereto until a crystalline solid [5] forms. The solid is collected, washed with ether and dried. If desired the solid can be recrystallized. Examples of various acids which can be used to prepare salts of [4] are provided in Example 9.

A reaction scheme for the preparation of DHE, its analogues and salts is shown in Fig. 14. Like etorphine, the starting material is thebaine and the first reaction with methyl vinyl ketone is identical to form the 6,14-endo-etheno derivative of the thebaine [2]. However, [2] is first subjected to catalytic hydrogenation and recovery to yield the 6,14-endo-etheno derivative of thebaine [3] before reaction with a Grignard reagent to produce [4]. The remaining synthetic steps proceed as described above for synthesis of etorphine. Hence, for DHE and related compounds, [2] is hydrogenated to produce, [3]; [3] is reacted with a Grignard reagent to produce [4]; and [4] is reacted with strong base to produce [5], the free base DHE or a related analog. Finally, [5] is reacted with an acid as described above to produce [6].

For DHE and related compounds, the R group of the Grignard reagent is lower alkyl as defined herein before. When R is n-propyl, then [5] of Fig. 14 is DHE. The preferred R group for DHE and an analogues thereof are n-propyl and i-amyl, respectively.

Another aspect of the invention is directed to pharmaceutical compositions of the opioid compounds of the present invention including dihydroetorphine and its analogues, etorphine and its analogues, ohmefentanyl as well as pharmaceutically acceptable salts of any of the foregoing compounds.

Dosage forms (compositions) suitable for administration can contain from about 10 μg to about 1000 μg of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered sublingually in solid dosage forms, such as capsules, tablets, and powders, or be administered parenterally in sterile liquid dosage forms.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Yet another aspect of this invention provides a pharmaceutical composition which comprises a low- or non-addictive opioid analgesic, or a pharmaceutically acceptable salt thereof, in admixture with naloxone which is an opioid antagonist. The low- or non-addictive opioid analgesics are those compounds as provided herein, e.g. etorphine, DHE, ohmefentanyl and the like, in amounts as provided herein.

The subject compositions are thus useful to avoid diversion or abuse of take-home preparations of solid forms, e.g. tablets of low- or non-addictive opioid analgesics

administered orally or sublingually to uses other than detoxification or severe pain relief. Since naloxone has low oral or sublingual bioavailability, an amount of naloxone can be introduced into the preparations that has no effect when taken orally or sublingually but antagonizes the effect of the low or non-addictive opioid analgesic, e.g. DHE, when the preparation is dissolved in water and injected. The amount of naloxone can be readily determined by one of ordinary skill in the art.

A still further aspect of this invention provides a pharmaceutical composition which comprises a low- or non-addictive opioid analgesic, or a pharmaceutically acceptable salt thereof, in admixture with a "replacement opioid". These compositions are useful for treating chronic or acute pain as well as opioid addictions. The dosages appropriate for each use can be readily determined by one of ordinary skill in the art. The low or non-addictive opioid analgesics include the compounds provided herein, e.g. etorphine, DHE, ohmefentanyl and the like, in amounts as provided herein. These pharmaceutical compositions are provided in formulations as described above.

The "replacement opioid" is a bimodally-acting opioid that has both inhibitory and excitatory effects on opioid receptors. The replacement opioid is formulated into compositions in an amount effective to (partially or wholly) relieve or suppress the withdrawal symptoms of opioid addiction, or an amount to alleviate pain. The amount of the low- or non-addictive opioid analgesic in these compositions is likewise that amount necessary to provide relief or suppression of withdrawal symptoms or to alleviate pain when used with a replacement opioid. One of ordinary skill in the art can readily determine a suitable ratio and dosage of analgesic and replacement opioid.

For example, suitable dosage forms for administration can contain from about 10 to about 1000 μg of the analgesic. When the analgesic is DHE, a preferred formulation for a

sublingual dosage contains about 20 μg to about 40 μg DHE, or the corresponding equivalent of a salt thereof, per tablet. A preferred formulation for an injectable dosage form contains about 20 μg to about 100 μg of DHE or the corresponding equivalent of a salt thereof.

Similarly, suitable dosage forms for administration of the replacement opioid can contain an amount which provides from about 5 mg to about 100 mg per day to the patient. Preferred replacement opioids include morphine, methadone, fentanyl and buprenorphine.

In a preferred embodiment, these pharmaceutical compositions contain DHE, or a pharmaceutically acceptable salt thereof such as DHE hydrochloride, and either methadone or morphine. Methadone is preferred for treating drug addiction whereas morphine is preferred for treating pain.

The examples serve to illustrate the present invention and are not to be used to limit the scope of the invention.

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## Example 1

### SELECTIVE INHIBITORY BUT NOT EXCITATORY EFFECT OF ETORPHINE AND DIHYDROETORPHINE ON THE ACTION POTENTIAL DURATION OF SENSORY DORSAL ROOT GANGLION NEURONS IN CULTURE

Tissue culture: The experiments were carried out on dorsal root ganglion (DRG) neurons in organotypic explants of spinal cord with attached DRGs (from 13-day-old fetal mice) after 3 to 5 weeks of maturation in culture. The DRG-cord explants were grown on collagen-coated coverslips in Maximow depression-slide chambers. The culture medium consisted of 65% Eagle's minimal essential medium, 25% fetal bovine serum, 10% chick embryo extract, 2 mM glutamine and 0.6% glucose. During the first week in vitro, the medium was supplemented with nerve growth factor (NGF-7s) at a concentration of about 0.5 μg/ml to enhance survival and

growth of the fetal mouse DRG neurons.

Electrophysiological recordings: The culture coverslip was transferred to a recording chamber containing about 1 ml of Hanks' balanced salt solution supplemented with 5 mM $Ca^{2+}$ and 5 mM $Ba^{2+}$ (BSS) to provide a prominent baseline response for pharmacological tests. Intracellular recordings were obtained from DRG perikarya selected at random within the ganglion with micropipette probes. The micropipettes were filled with 3M KCl (resistance about 60-100 megohms) and were connected via a chloridized silver wire to a neutralized input capacity preamplifier (Axoclamp 2A) for current clamp recording. After impalement of a DRG neuron, brief (2 msec) depolarizing current pulses were applied via the recording electrode to evoke action potentials (at a frequency of 0.1 hz). Recordings of the action potentials were stored on a floppy disc using the p-clamp program (Axon Instruments) in a microcomputer (IBM AT-compatible).

Drug test: Drugs were applied by bath perfusion with a manually operated push-pull syringe system at a rate of 2-3 ml/min. Perfusion of test agents was begun after the action potential and the resting potential of the neuron reached a stable condition during >4 min pretest periods in control BSS. Opioid-mediated changes in the APD were considered significant if the APD alteration was >10% of the control value for the same cell and was maintained for the entire test period (about 5 min). The APD was measured as the time between the peak of the APD and the inflection point on the repolarizing phase.

Opioid Responsiveness: The opioid responsiveness of DRG neurons was analyzed by measuring opioid-induced alterations in the APD of DRG perikarya. DRG neurons in DRG-cord explants were examined for sensitivity to acute application of etorphine or dihydroetorphine at fM to μM concentrations. None of the cells (n=12) showed APD shortening or prolongation in 1 fM etorphine. However, naloxone-reversible APD shortening was observed in 25% of the cells

(n=8) after application of pM and nM concentrations of etorphine and in 100% of the cells (n=7) after application of μM concentrations of etorphine (Figs. 3 and 4). None of the DRG neurons tested with different concentrations of etorphine (n=13) showed APD prolongation.

These results are in sharp contrast to other mu, delta or kappa opioids (e.g. morphine, methadone, DAGO, DPDPE, DADLE, dynorphin (amino acids 1-13) or (amino acids 1-17) and U-50,488H), each of which show bimodal action such that low concentrations (<nM) evoked excitatory APD-prolonging effects and higher concentrations (~μM) evoked inhibitory APD-shortening effects on many DRG neurons (Fig. 4; Table 1). For Fig. 4, data were obtained from 11 neurons for etorphine test, half of which were tested with all four concentrations of etorphine (from fM to μM).

Like etorphine, electrophysiologic tests with dihydroetorphine (over fM-μM ranges) on DRG neurons (n=15) showed concentration-dependent inhibitory APD shortening effects, with threshold at fM-pM, and no evidence of excitatory APD prolonging effects (Fig. 4).

TABLE 1

Alteration of action potential duration of dorsal root ganglion neurons treated with high and low concentrations of opioids

| | Alteration of Action Potential Duration | |
|---|---|---|
| | Opioid at low concentration < nM | Opioid at high concentration μM |
| Morphine | Prolongation | Shortening |
| DAGO | Prolongation | Shortening |
| DADLE | Prolongation | Shortening |
| DPDPE | Prolongation | Shortening |
| U-50,488H | Prolongation | Shortening |
| Dynorphin 1-13 | Prolongation | Shortening |
| Dynorphin 1-17 | Prolongation | Shortening |
| Met-enkephalin | Prolongation | Shortening |
| Leu-enkephalin | Prolongation | Shortening |
| ß-endorphin | Prolongation | Shortening |
| Methadone | Prolongation | Shortening |
| Fentanyl | Prolongation | Shortening |
| Levorphenol | Prolongation | Shortening |
| Thebaine | Prolongation | Shortening |
| Etorphine* | Shortening | Shortening |
| Dihydroetorphine* | Shortening | Shortening |

* Selectively activate inhibitory (APD shortening), but not excitatory, opioid receptor-mediated functions.

Example 2

ENHANCED INHIBITORY EFFECT OF ETORPHINE ON CHRONIC OPIOID-TREATED, ADDICTED SENSORY NEURONS THAT HAD BECOME SUPERSENSITIVE TO THE EXCITATORY EFFECTS OF BIMODALLY ACTING OPIOID AGONISTS AND TO NALOXONE

Drug tests: Mouse DRG-cord explants, grown for >3 weeks as described in Example 1, were chronically exposed to the bimodally acting (excitatory/inhibitory) delta/mu opioid agonist, DADLE (3 μM) or morphine (1 μM) for 1 week or longer. Electrophysiological recordings were made as in Example 1.

Results: After such chronic exposure, DRG neurons are supersensitive to the excitatory effects of opioids (Crain & Shen 1992a; Shen & Crain, 1992). Whereas pM-nM Dyn (amino acid 1-13) is generally required to prolong the APD of naive DRG neurons (Fig. 4), fM levels and lower are effective at prolonging the APD after chronic opioid treatment (Fig. 5, traces 1-4). In contrast, acute application of etorphine to chronic DADLE-treated neurons effectively shortened the APD of the same DRG neurons that showed supersensitive excitatory responses to low concentrations of bimodally-acting opioids (Fig. 5, traces 6-9). Furthermore, the inhibitory APD-shortening effect of etorphine on DRG neurons appears to be significantly enhanced. While pM etorphine was effective in shortening the APD of 25% of the DRG neurons tested in naive explants (Figs. 3 and 4), this low opioid concentration was effective in all of the chronic DADLE-treated DRG neurons tested in the presence of 1 μM DADLE (n=4; Fig. 5, traces 5 and 6). This same low concentration of etorphine (pM) was effective in 71% of the chronic morphine-treated (1 μg/ml) DRG neurons tested in the presence of 1 μg/ml morphine (n=7). Dose response tests on chronic DADLE-treated DRG neurons showed, in fact, that the magnitude of the APD was progressively shortened when the acute etorphine concentration was increased sequentially from 1 fM to 1 μM (Fig. 5, traces 6-9).

The opioid antagonist, naloxone (nM-μM), does not alter

the APD of naive DRG neurons (Crain & Shen 1992a, b). In contrast, after chronic opioid, such as DADLE treatment, acute application of low concentrations of naloxone prolongs the APD of sensory neurons (Crain et al, 1992b; Shen & Crain, 1992). The naloxone-induced excitatory APD-prolonging effect on chronic opioid-treated DRG neurons is shown in Fig. 6, traces 1 and 2. Acute application of low concentrations of etorphine (pM-nM) effectively blocks the naloxone-induced APD prolongation of DRG neurons (n=3; Fig. 6, traces 3 and 4) whereas bimodally acting opioids are ineffective.

Since etorphine and dihydroetorphine elicit potent inhibitory effects on naive sensory neurons even when applied at extremely low (pM) concentrations and show no signs of concomitantly activating excitatory opioid receptors on these cells, these in vitro electrophysiologic analyses predict that application of etorphine and dihydroetorphine in vivo at the relatively low doses required to produce analgesia (<1,000 times lower than morphine) are not addictive even after sustained application for treatment of chronic pain.

## Example 3

### SUPPRESSION OF WITHDRAWAL SYMPTOMS BY DHE IN MORPHINE-DEPENDENT RATS

Morphine-dependent rat model: Wistar rats of both sexes, 120-150 g body weight, were administered morphine subcutaneously (s.c.) twice a day (8:00 a.m., 4:00 p.m.) starting at a dose of 20 mg/kg/day, with an increment of 20 mg/kg/day for 5 consecutive days until the final dose reached 100 mg/kg/day.

Naloxone (NLX) precipitation for the scoring of withdrawal symptoms: 3-4 hrs after administering the last dose of morphine (or other test drug(s)), withdrawal symptoms of morphine-dependent rats were precipitated by intraperitoneal (i.p.) injection of naloxone (4 mg/kg). Naloxone-induced withdrawal symptoms were monitored for 1 hr

thereafter and scored according to the method of Wei et al, 1973.

Animal groups: After 5 days of morphine addiction, the animals were divided into 7 groups according to Table 2. Each group contained 5-6 rats.

Groups 1, 2, and 3 received 20 mg/kg morphine (4 times the $ED_{50}$ for analgesia), 9 mg/kg methadone (9 times the $ED_{50}$ analgesia) or 6 μg/kg DHE (12 times the $ED_{50}$ analgesia) by i.p. injection, respectively. These opioid agonists were injected 15-30 min before naloxone precipitation was initiated. After the naloxone withdrawal test was completed, groups 1, 2, and 3 were continued on morphine 100 mg/kg (s.c.) for another 4 consecutive days. A second naloxone precipitation test was given on the 4th day but only saline was administered (i.p.) prior to naloxone.

The first naloxone precipitation test was performed on the animals of groups 4, 5, and 6 in the same manner as for groups 1-3, except the administration of opioid agonists 15-30 min prior to the naloxone test. For the second naloxone test, groups 4, 5 and 6 received 100 mg/kg morphine (s.c.) twice a day, 3 μg/kg DHE 4 times a day, or 6 mg/kg methadone, 4 times a day, instead of morphine at 100 mg/kg, for 4 days, respectively. The second naloxone test was performed as above on the 4th day.

After the first naloxone precipitation test, Group 7 animals were given saline (s.c.) as control for 4 days before the second naloxone test.

The body weight of the animals was monitored during the entire period.

Results: One to 2 min after intraperitoneal injection of naloxone, the morphine-dependent rats began to show naloxone induced withdrawal symptoms with a peak response occurring within 15 min. An hour later the body weight of the animals was greatly reduced. Intraperitoneal injection of morphine (20 mg/kg), DHE (6 μg/kg) or methadone (9 mg/kg) prior to the administration of naloxone suppressed the

naloxone induced withdrawal symptoms of the rats. No significant differences in suppressing effect were detected among these three opioid substitutes. For morphine, DHE and methadone, prevention of body weight loss was 43.5%, 49.8% and 48.15%, respectively, and suppression of other withdrawal symptoms was scored as 45.5%, 63.7% and 49.4%, respectively.

After naloxone precipitation, the body weight of the dependent rats continued to decrease. The loss of body weight reached its maximum 24 h after naloxone precipitation. A gradual weight recovery was achieved by 90 h.

Subcutaneous injection of morphine, DHE or methadone was given for several days after the first naloxone precipitation test. The loss of body weight of morphine-dependent rats was found to be reduced in all three groups treated with opioid agonists. Subcutaneous administration of morphine (one hour after naloxone precipitation) reversed the body weight loss in 3 hours, with occasional weight gain in some of the rats. A complete recovery of weight loss was observed 48 h later. The effect of subcutaneous injection of DHE or methadone on body weight loss was not as dramatic as with morphine. However, both opioids did prevent further body weight loss. When compared with the untreated control group (saline injected), the effect of both DHE or methadone on body weight loss was highly significant (Fig. 7).

After the first naloxone precipitation test, some of the animals continued to be maintained on morphine (s.c.). Four days later, a second naloxone test was given. The second naloxone test resulted in more severe withdrawal symptoms relative to the first test. In contrast, in those animals that were treated with DHE (s.c., 4 days) instead of morphine, the second naloxone test failed to precipitate any withdrawal symptoms except minor loss in body weight. In the animals maintained with methadone (s.c., 4 days), the

second naloxone injection precipitated less severe withdrawal symptoms in comparison to the morphine group, yet more severe when compare with the DHE group (Figs. 8 and 9).

Table 2

Animal Groups Used to Test the Suppression of NLX-induced Withdrawal Symptoms by Different Opioids

| Animal Groups | Development of Morphine Dependence 5 days | Pretreatment (15-30' prior to 1st test) | 1st NLX Test | Continued Maintenance with Opioids 4 days | Pretreatment (15-30' prior to 2nd test) | 2nd NLX Test |
|---|---|---|---|---|---|---|
| 1 | Morphine 20—>100 mg/kg | Morphine (20 mg/kg) | NLX | Morphine 100 mg/kg | Saline | NLX |
| 2 | Same as 1 | Methadone (9 mg/kg) | NLX | Same as 1 | Saline | NLX |
| 3 | Same as 1 | DHE (6 µg/kg) | NLX | Same as 1 | Saline | NLX |
| 4 | Same as 1 | -- | NLX | Morphine 50 mg/kg | -- | NLX |
| 5 | Same as 1 | -- | NLX | DHE 3 µg/kg | -- | NLX |
| 6 | Same as 1 | -- | NLX | Methadone 6 mg/kg | -- | NLX |
| 7 | Same as 1 | -- | NLX | Saline | -- | NLX |

Example 4

ANTI-ADDICTIVE EFFECTS OF DHE TREATMENT OF MORPHINE-DEPENDENT MONKEYS

Morphine-dependent monkey model: Seven male rhesus monkeys (Macaca mulatta, 3.4-5 kg) were injected with morphine (s.c.) twice a day (8:00 a.m., 4:00 p.m.), starting at a dose of 10 mg/kg/day and increasing the dose by increments of 5 mg/kg/day every third day until the dosage reached 50 mg/kg/day on the 24th day. This dosage was continued for another 10 days prior to performing drug tests.

Stage 1 drug tests: The monkeys were randomly divided into 2 groups. At 24 h after withdrawal of morphine, Group A (4 animals) received 3 µg/kg DHE (s.c.) every 3 h. The interval between DHE administration was increased gradually so that by the 3rd day, DHE was only given twice a day, and then stopped for 2 days of observation. Group B (3 animals) was treated in the same manner as group A except, this group received saline instead of DHE. After completion of these

tests, all the animals were treated with morphine for 12 consecutive days by administration of 50 mg/kg/day morphine (s.c.) twice a day. The test was repeated except the Group A monkeys received the saline controls and the Group B monkeys received the DHE treatment. Withdrawal symptoms of the animals were observed and scored according to Deneau & Seevers (1964), during the entire experimental period.

Sixteen hours after withdrawal of morphine, withdrawal symptoms began to appear in the morphine-dependent monkeys. Symptoms were moderate at first and included yawning, salivation, agitation and fear. These signs became more severe as time went on. Within 20-60 h after withdrawal of morphine, the animals' withdrawal symptoms included vomiting, tremor, teeth-gritting on chain, eye closing, lying on its side and dyspnea. All these symptoms are indicative of extreme agitation. After 60 h these symptoms gradually subsided. By 120 h after withdrawal of morphine, some moderate withdrawal symptoms were still detected (Fig. 10). One week later all the withdrawal symptoms had disappeared.

In sharp contrast, all of these withdrawal symptoms were completely suppressed by DHE one minute after its administration (3 μg/kg. s.c.). Two and a half to three hours later, withdrawal symptoms reappeared which were again suppressed by another dose of DHE (Fig. 11). This suppressing effect of DHE on morphine withdrawal symptoms was observed with each monkey. DHE continued to be effective at suppressing withdrawal symptoms for 3-4 days with repeated injections at 2.5-3 h intervals. Discontinuation of DHE injection at 80 h after morphine withdrawal did not trigger any withdrawal symptoms, indicating that the animals had not become dependent on DHE during this substitution treatment.

Stage 2 drug tests: After the stage 1 experiments, all 7 monkeys were administered morphine (s.c.) at a dose of 50 mg/kg/day for 7 days. The morphine-addicted monkeys were

then randomly divided into 3 groups. Group 1 was maintained with s.c. injection of 25 mg/kg morphine twice a day for 9 days. Group 2 was substituted with DHE by s.c. injection of 3 μg/kg DHE (equi-analgesic dose) four times a day for 4 days, of 1.5 μg/kg DHE three times a day for 2 days and then twice a day for 3 days. Group 3 was substituted with methadone by s.c. injection of 6 mg/kg methadone (equi-analgesic dose) four times a day for 4 days, of 3 mg/kg three times a day for 2 days and then twice a day for 3 days.

Sixteen hours after the last injection of opioid, each animal was precipitated with naloxone (1 mg/kg, s.c.) to evaluate the severity of naloxone withdrawal symptoms for 1 day. Seven days later, another naloxone precipitation test was performed on these monkeys for 1 day. After completion of all tests, 3 monkeys were randomly selected for morphine addiction (25 mg/kg, s.c., twice a day for 7 days). Naloxone precipitation tests were performed twice on these 3 monkeys, the first trial given after the last injection of morphine and the second trial given 7 days thereafter.

Since the action period of DHE and methadone is relatively short, some moderate withdrawal symptoms appeared during the 6 hr intervals between injections on the first 3 days. After these 3 days, the withdrawal symptoms became milder and gradually disappeared.

Naloxone precipitation tests were carried out after 9 days of substitution treatment with DHE or methadone. For the monkeys maintained on morphine, naloxone injection precipitated a series of withdrawal symptoms after 15 sec. These symptoms included squeaking, coughing, rolling, tremor, vomiting, agitation, teeth-gritting on chain, dyspnea, and finally lying down on the ground. The animals recovered by 7 days later. The monkeys substituted with methadone showed moderate naloxone withdrawal symptoms including yawning, placing hands on the belly, tremor of extremities, frequent teeth-gritting on chain and agitation.

However, those animals substituted with DHE showed no change in behavior both before and after naloxone precipitation. Table 3 shows the scores of naloxone withdrawal symptoms from the 3 different groups of monkeys. Once morphine was fully excreted from the body (7 days after withdrawal), naloxone no longer precipitated any withdrawal symptoms.

The naloxone precipitation test was used to evaluate whether these animals were dependent on morphine or had become dependent on the substitution opioid.

Fig. 12 illustrates the variations in the scores of withdrawal symptoms in monkeys after DHE or methadone substitution relative to compulsive withdrawal. In the compulsive withdrawal group (upper trace) that withdrawal symptoms reached a maximal score during the first several days, but returned to zero by 7 days after abrupt morphine withdrawal. On day 9, naloxone no longer precipitated any withdrawal symptoms. For the methadone substitution group (middle trace), the withdrawal symptoms during the first several days were partially suppressed. On day 9, naloxone precipitated withdrawal symptoms, suggesting that the animals have already switched to methadone dependence. For DHE substitution group (lower trace) only minor withdrawal symptoms were observed. Naloxone precipitation tests on day 9 did not trigger any withdrawal symptoms. These results indicate that DHE is an ideal low- or non-addictive substitution drug for treatment of opioid abstinence problems.

Table 3

Scores of naloxone withdrawal syndromes in morphine dependent monkeys with or without DHE or methadone substitution treatment

| Treatment[a] (n) | Scores of withdrawal syndromes (X ± SD) | |
|---|---|---|
| | 1st naloxone precipitation[b] | 2nd naloxone precipitation[c] |
| morphine (4) | 49.0 ± 2.2 | 1.5 ± 1.0 |
| DHE (3) | 2.0 ± 1.0*** | 1.0 ± 1.0 |
| methadone (3) | 17.0 ± 4.6*** | 1.3 ± 1.2 |

[a] Daily treatment dosages were 50 mg/kg morphine (divided into 2 subdoses), 12 μg/kg DHE (divided into 4 subdoses) decreased to 3 μg/kg (divided into 2 subdoses), and 24 mg/kg methadone (divided into 4 subdoses) decreased to 6 mg/kg (divided into 2 subdoses).

[b] The first naloxone precipitation test was performed 16 h after the last injection of opioid.

[c] The second naloxone precipitation test was performed 7 days after the last injection of opioid.

*** $p < 0.01$, compared to the morphine group. For the DHE group compared to the methadone group, then $p<0.01$.

Example 5

DHE ELICITS POTENT LOW- OR NON-ADDICTIVE ANALGESIA IN ACUTE AND CHRONIC PAIN PATIENTS

The results in the first stage clinical trial showed that none of the 20 volunteers had euphoria feeling after DHE administration through sublingual route at 60 μg single dose. At high dosage (e.g. > 1 mg per day), dizziness, nausea, vomiting and lethargy appeared. The results from second stage clinical trial demonstrated that DHE can effectively relieve postoperative pain and pain caused by terminal stage of cancer. The effective rate of 730 cases that have complete medical records was 97.6%. Among them, the effective rate of acute pain in patients from

departments of surgery, obstetric and gynecology approached nearly 100%. The effective rate for relief of chronic severe pain and terminal stage of cancerous pain was 90-95%. The clinical data indicate that the analgesic effect of DHE is substantial with only mild side effects. DHE treatment was effective in those terminal stage cancer patents that were unresponsive to morphine or pethidine (demerol) treatment. No cross tolerance to DHE was found in these patients. Long-term use of DHE can result in tolerance; however, the degree of tolerance is less than that observed with morphine or pethidine.

Clinical treatment with DHE has been conducted in more than one hundred thousand patients in China. As an analgesic, the main disadvantage of DHE is its short action period (about 3-4 hours). Compared with morphine, DHE has high analgesic effect and low addictivity, whereas morphine has relatively low analgesic effect and high addictivity. During many years of trials using DHE as an analgesic, no cases of drug abuse were ever reported. This phenomena may be attributed to the strict regulation of DHE treatment. The medication period for ordinary pain is typically limited to 1 week; whereas, for patients with terminal cancer pain, the treatment period is much longer. Although some of the patients became tolerant to DHE after long-term use, there is a slight chance that a few patients may become addicted to the drug after long-term use (e.g., > 6 months).

Example 6

DHE SUBSTITUTION TREATMENT SUPPRESSES WITHDRAWAL SYMPTOMS IN OPIATE ADDICTS WITHOUT CONCOMITANT DHE ADDICTION

General protocol: Institution of DHE therapy as a substitute drug began with a sufficient dose on days 1-3 to suppress completely the withdrawal symptoms. On days 4-7, the dosage was reduced and by days 8-10 the DHE substitution therapy was terminated. This protocol was followed because (1) withdrawal symptoms are most severe during the first 3 days after abrupt withdrawal of heroin or other addictive opioid; (2) withdrawal symptoms gradually decline and disappear after 7-10 days; and (3) consecutive use of DHE as a substitution agent for 7-10 days does not produce any self-dependence.

DHE administration: More than 300 cases of chronic heroin users were treated for 7-10 days with DHE in 10 hospitals in China. DHE was administered either sublingual in tablet form (40 μg) or by intramuscular injection (20 μg) or by intravenous dripping (20 μg). The tablet form was used more often. At the onset of withdrawal symptoms, sublingual administration of 1-2 tablets (20-40 μg) of DHE effectively suppressed the symptoms. Sustained suppression of withdrawal symptoms required repeated DHE medication every 2-4 h. Total dosage was adjusted according to the severity of the withdrawal symptoms. Typically after 4 days of DHE medication, the dosage required to suppress withdrawal symptoms was generally reduced. The entire course of DHE substitution was generally 7 days. (In one instance of overdose of DHE, respiratory side effects occurred.)

For addicts whose withdrawal symptoms were so severe and violent that sublingual medication was not enough to subdue them, intramuscular injection of DHE (20 μg) gave instant relief. The addict generally became quiet and cooperative. However, to maintain the therapeutic effect, it was necessary to administer DHE by intravenous dripping

(100 μg in 500 ml glucose saline for 6-10 hr). The transfusion rate depended upon the severity of symptoms: the drip rate was increased when the patient showed sign of restlessness or decreased when the patient was quiet and complained of lethargy. In severe cases, intravenous transfusion of DHE was maintained for 3-4 days with progressive decrease in dosage. On day 4 or 5, intravenous dripping of DHE was converted to sublingual DHE administration and terminated on day 7. Occasionally treatment was prolonged to 8-9 days, but never more than 10 days to prevent possible occurrence of dependence. Hence, by using this optimal 7-10 day treatment period DHE is effectively employed as a substitute for drug addiction therapy.

The effectiveness of DHE substitution therapy was evaluated on day 10 by the naloxone precipitation test (0.4-0.8 mg naloxone, intramuscular injection) and urine analysis of the residual amount of opioid. The treatment course was considered successful if both tests were negative.

One of the primary advantages of DHE over methadone substitution therapy is the early onset of DHE effectiveness. Withdrawal symptoms were markedly relieved after 10-20 min of sublingual administration or 5 min of intramuscular injection of DHE. In contrast, with methadone substitution the first dose usually begins at 10 mg and is increased every hour until the therapeutic effect is achieved. Such treatment can last from one to several hours before symptomatic relief. This period is intolerable to a patient with severe withdrawal symptoms. Furthermore, methadone substitution therapy often results in the rebound of withdrawal symptoms, suggesting dependence on methadone.

In contrast, cessation of DHE administration generally proceeded smoothly. As with methadone, the side effects of DHE were negligible during substitution treatment for drug addiction. Since DHE is short acting (only 2-4 hr), frequent administration may be necessary. To avoid this,

intravenous dripping of DHE is recommended. However, intravenous administration can only be prescribed in the hospital and is not applicable to the ordinary drug rehabilitation clinic. Since methadone is effective orally (once or twice a day), one alternative is to combine DHE and methadone treatments. For example, DHE is used initially for swift control of the withdrawal symptoms and is then replaced by methadone to maintain the suppressing effect for 2-3 days. The treatment is then switched back to DHE on a decreasing dosage regime until DHE is no longer needed (usually another 5-10 days). This combined therapy is safe, pragmatic and convenient.

Example 7

According to the following synthetic route (Figure 15), dihydroetorphine hydrochloride was prepared using thebaine [Compound 1] as a starting material.

(a) Preparation of 7°-acetyl-6,14-endo-etheno-di77hydro-thebaine [Compound 2]

A mixture of thebaine (49.8 g, 0.16 mol, Compound 1) and methyl vinyl ketone (150 mL) was refluxed in a 250 mL round-bottomed flask for 1 hour. The excess ketone was distilled off under reduced pressure. Warm methanol (60 mL) was added to dissolve the thickened oil. Under cooling a crystalline product formed and it was filtered, washed with ice-cold methanol for 2-3 times and dried. The solid (56.6 g, yield 93%) was obtained, m.p. 120-122°C.

(b) Preparation of 7°-acetyl-6,14-endo-ethano-tetrahydro-thebaine [Compound 3]

A mixture of compound [2] (19.1 g, 0.05 mol), 10% Pd/C (4 g) and absolute alcohol (200 mL) was catalytically hydrogenated under the hydrogen pressure of 40-50 kg/cm² at 55-60°C with stirring for 8-12 hours. The catalyst was filtered off and filtrate was concentrated. After cooling, the crystalline product was collected and washed with absolute alcohol. The white solid (15.7 g, yield 82%) was obtained, m.p. 135-137°C.

(c) Preparation of 7"-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethano-tetrahydrothebaine [Compound 4]

The Grignard reagent was prepared by the reaction of bromopropane (127.9 g, 1 mol) and magnesium (24.3 g, 1 mol) in 1100 mL of absolute ether. The compound [3] (99.7 g, 0.26 mol) in 1100 mL of benzene was added dropwise with vigorous stirring and under reflux. The reaction mixture was stirred and refluxed for another 2 hours. A saturated ammonium chloride solution was poured into the mixture, which separated the organic layer. The aqueous layer was extracted with ether for several times. The combined ether extract was washed with water until the washings became neutral. The extract was dried over anhydrous magnesium sulphate. After evaporating the solvent, the crude product was recrystallized from absolute alcohol. The white solid (75-79 g, yield 67-71%) was obtained, m.p. 184-186°C.

(d) Preparation of 7"-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethano-tetrahydrooripavine [Compound 5]

A mixture of compound [4] (85.5 g, 0.2 mol), diethylene glycol (1700 mL) and potassium hydroxide (616 g) was placed in a four-necked flask. After the low-boiling substance was distilled off under a nitrogen stream, the reaction mixture was heated at an internal temperature of 200-210°C and stirred for 14-16 hours. The resultant mixture was poured into 10 L of water to dissolve it. A suitable amount of ammonium chloride was added until the solid separated out completely. The solid was filtered, washed with water until the washings became neutral, dried and extracted with absolute ether. After ether was distilled off, the crude product was recrystallized from methanol. The pure compound (54-58 g, yield 66-70%) was obtained, m.p. 204-206°C.

Chemical and Spectral Analysis: $C_{25}H_{33}NO_4$

| | | | | | | |
|---|---|---|---|---|---|---|
| Calc:% | C | 72.63 | H | 8.53 | N | 3.38 |
| Found:% | C | 72.40 | H | 8.65 | N | 3.22 |
| | $M^+$: | 413 | | | | |

IR (KBr): $\tau$ ($cm^{-1}$) 3528, 3478, 3314, 3186 (OH);

1213 (Ar-O); 1110 (tert. alc. C-O); 3040 (Ar-H); 1633, 1612, 1498 (Ar C=C); 1152 (C-O-C); 1283,1085, 882 (Ar-O-C); 2790, 2764 (=N-$CH_3$); 820 (Ar-H).

$^1$HNMR: ($CDCl_3$, 400 MHz, $\delta$ ppm)
0.78 (m, 1H, 8αH); 0.87 (m, 3H, 25$CH_3$); 1.02, 2.66 (m, 2H, 18$CH_2$); 1.07 (m, 1H, 8ßH); 1.32 (s, 3H, 22$CH_3$); 1.47 (m, 2H, 23$CH_2$); 1.40 (m, 2H, 24$CH_2$); 1.64, 2.00 (m, 2H, 15$CH_2$); 1.76, 1.90 (m, 2H, 19$CH_2$); 1.78 (m, 1H, 7CH); 2.21 (d, 1H, 10αH); 2.26, 2.44 (m, 2H, 16$CH_2$); 2.30 (s, 3H, 17$CH_3$); 2.65 (m, 1H, 9CH); 3.09 (d, 1H, 10ßH); 3.46 (s, 1H, 30H); 3.55, (s, 3H, 20$CH_3$); 4.37 (s, 1H, 5CH); 5.32 (s, 1H, 26OH); 6.50 (d, 1H, 1CH); 6.67 (d, 1H, 2CH).

$H_1,H_2$ = 8 Hz, $H_{10ß},H_{10α}$ = 18.3 Hz, $H_{25},H_{24}$ = 6 Hz, $H_{15},H_{15}$ = 11.7 Hz, $H_9,H_{10α}$ = 6.5 Hz.

$^{13}$CNMR: ($CHCl_3$, 100 Hz, $\delta$ ppm)
$C_{25}$=14.65, $C_{24}$=15.86, $C_{19}$=17.87, $C_{10}$=21.94, $C_{22}$=23.36, $C_8$=29.73, $C_{18}$=31.70, $C_{15}$=35.38, $C_{14}$=36.00, $C_{23}$=43.44, $C_{17}$=43.70, $C_{16}$=45.13, $C_7$=45.40, $C_{21}$=46.30, $C_{20}$=52.62, $C_9$=61.23, $C_{13}$=76.13, $C_6$=30.38, $C_5$=96.95, $C_1$=116.76, $C_2$=119.35, $C_{11}$=127.57, $C_{12}$=131.92, $C_3$=137.72, $C_4$=145.67.

(e) Preparation of dihydroetorphine hydrochloride [Compound 6]

The free base [5] (14 g, 0.034 mol) was dissolved in a mixed solvent of absolute alcohol (400 mL) and absolute ether (640 mL). An amount of ether saturated with dried hydrogen chloride was added dropwise until the solution became acidic (pH = 2). After adding 200 mL of absolute ether, a crystalline solid formed. The white solid was

collected, washed with absolute ether and dried. The desired final product (14-14.5 g, yield 92-94%) was obtained, m.p. 297-298°C, $[\alpha]^{20}$ -65.

Chemical and Spectral Analysis: $C_{25}H_{35}NO_4.HCl$

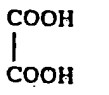
| | | | | | | |
|---|---|---|---|---|---|---|
| Calc:% | C | 66.72 | H | 8.06 | N | 3.11 |
| Found:% | C | 66.70 | H | 8.15 | N | 3.09 |

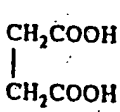
Example 8

PREPARATION OF VARIOUS DIHYDROETORPHINE (DHE) SALTS AND ANALYSIS OF DURATION AND POTENCY OF THE ANALGESIC EFFECTS THEREOF

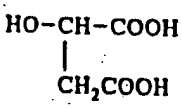
A total of 26 dihydroetorphine (DHE) salts were prepared according to step (e) of Example 7 except the various acids listed below were substituted for HCl.

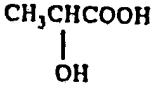
I. Structures of 26 acids employed to form derivatives of dihydroetorphine salts

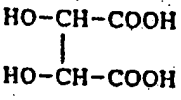
*1.


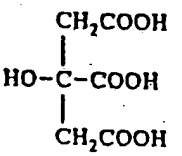
*2.


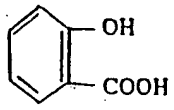
*3. $CH_3COOH$

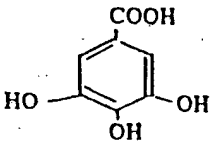
*4.


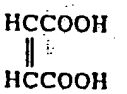
5.


6.


*7.


8. 

9. 

*10. 

11. $CH_2CH=CHCH=CHCOOH$

12. $CH_3(CH_2)_{10}COOH$

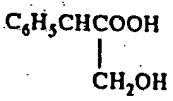
13. $CH_3(CH_2)_{14}COOH$

14. $CH_3(CH_2)_{16}COOH$

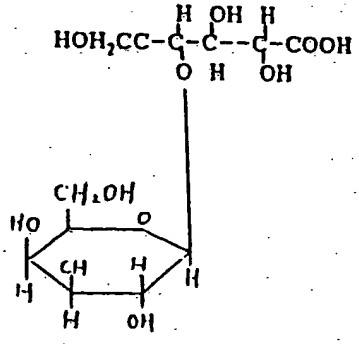
*15. $C_6H_5CH(OH)COOH$

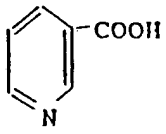
*16. $(C_6H_5)_2C(OH)COOH$

17. $C_{76}H_{52}O_{46}$

18. 

19. $C_6H_5SO_3H$

20. 

21. 

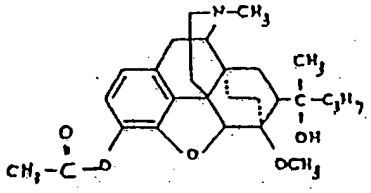
22. $CH_3CHCOOH$ (OH)

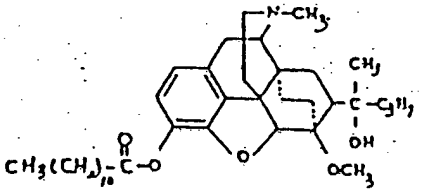
23. $C_6H_4(NH_2)(SO_3H)$

*24. HBr

*25. $HO_2CCH(NH_2)CH_2COOH$

*26. $HO_2CCHNH_2(CH_2)_2COOH$

27. 

28. 

* Preliminary screening tests have been done.

A "mouse hot plate" (55°C ± 0.5°C) method as previously described (Huang and Qin, 1982) was used to score % analgesia to measure the potency of each DHE salt which was injected to animals subcutaneously. The $ED_{50}$ (the dose that gives rise to 50% analgesia as calculated by the following formula) was measured for the DHE salts shown in Table 4.

$$\text{Analgesia \%} = \frac{\text{Pain threshold (sec) after administration} - \text{Pain threshold (sec) before administration}}{60 - \text{Pain threshold (sec) before administration}}$$

A dose of 5 $ED_{50}$ was used for each salt to measure the corresponding analgesic duration. "Analgesia %" was recorded at 90, 120 and 150 min after administration (Table 5).

In summary, an $ED_{50}$ (μg/kg) in the range of 0.50 to 2.0 was observed with the 12 DHE salts tested, indicating an equivalent level of analgesic effect conferred by these salts (see data presented in Table 4). Furthermore, except for acetyl DHE, DHE maleate, and DHE amygdalate, all DHE salts demonstrated an equivalent level of analgesia over a 120-150 min duration.

Table 4

Analgesic Effect of Various DHE Salts

| Salts of DHE* | $ED_{50}$ (μg/Kg) |
|---|---|
| DHE hydrochloride | 1.43 |
| Acetyl DHE (27) | 0.47 |
| DHE maleate (10) | 0.58 |
| DHE succinate (2) | 0.83 |
| DHE oxalate (1) | 0.68 |
| DHE acetate (3) | 0.51 |
| DHE malate (4) | 0.62 |
| DHE asparagate (25) | 1.12 |
| DHE glutamate (26) | 0.65 |
| DHE amygdalate (15) | 0.61 |
| DHE dibenzoylhydroxyl acetate (16) | 1.29 |
| DHE citrate (7) | 1.73 |

* The numbers following each salt correspond to the numbered compounds of Example 8

Table 5

| Salts of DHE | Analgesia % (x ± SD) | | |
|---|---|---|---|
| | 90 min | 120 min | 150 min |
| DHE hydrochloride | 42.39 ± 31.34 | 20.05 ± 10.91 | 28.49 ± 14.21 |
| Acetyl DHE | 5.96 ± 12.65 | 8.85 ± 9.47 | ND |
| DHE maleate | 15.84 ± 13.82 | ND | ND |
| DHE succinate | 26.16 ± 14.86 | 30.77 ± 42.35 | 32.26 ± 35.89 |
| DHE oxalate | 14.21 ± 6.37 | 19.35 ± 24.14 | ND |
| DHE acetate | 21.64 ± 10.11 | 29.54 ± 30.93 | ND |
| DHE malate | 26.92 ± 23.75 | 22.36 ± 19.29 | ND |
| DHE asparagate | 40.96 ± 37.28 | 35.94 ± 45.06 | 21.44 ± 35.68 |
| DHE glutamate | 29.33 ± 36.15 | 9.27 ± 12.57 | 14.09 ± 17.07 |
| DHE amygdalate | 29.15 ± 26.73 | 1.87 ± 2.97 | ND |
| DHE dibenzoylhydroxyl acetate | 45.01 ± 49.66 | 13.85 ± 17.49 | ND |
| DHE citrate | 56.12 ± 46.42 | 25.95 ± 37.46 | 14.27 ± 14.49 |

Example 9

The pharmaceutical preparations of dihydroetorphine hydrochloride include a parenteral injectable sterile solution and a sublingual tablet.

(a) Preparation of injectable dihydroetorphine hydrochloride

This injectable is a pharmaceutical preparation in a sterile aqueous solution. Its outward appearance is transparent and colorless. Each ampule contains 20 μg of said compound as the active ingredient in 1 mL of solution.

The prescription is shown as follows:

Dihydroetorphine hydrochloride 20 mg
0.001 N Hydrochloric acid q.s. 1000 mL

(b) Preparation of dihydroetorphine hydrochloride sublingual tablet

The outward appearance of the sublingual tablet is white. Each tablet contains 20 μg or 40 μg of said compound as active ingredient.

For example, the prescription for 10000 tablets at 40 μg per tablet is as follows:

Dihydroetorphine hydrochloride 400 mg

| | |
|---|---|
| Lactose:starch:mannitol:sucrose (3:1:3:3) | 600 g |
| Sodium carboxymethyl cellulose (1% aque sol'n) | 18 mL |
| Ethyl alcohol (50%) | 24 mL |
| Magnesium stearate | 6 g |

According to the above-mentioned prescription, a designated amount of dihydroetorphine hydrochloride was weighed and dissolved in 50% ethyl alcohol. This solution was added dropwise onto excipients under mechanical stirring to ensure uniformity. Meanwhile, 1% sodium carboxymethyl cellulose solution was added dropwise. The soft material thus formed was screened through a 20-mesh sieve and the same operation was repeated for 3 times. The product was then dried in an oven at 60°C. Magnesium stearate was added as a -lubricating agent for the tablets.

WE CLAIM:

1. An in vitro screening method for identifying a low- or non-addictive opioid analgesic which comprises identifying a compound which is capable of evoking an inhibitory effect on an opioid receptor-mediated function and which is not capable of evoking an excitatory effect on said function when said compound is present at a concentration ranging from about femtomolar (fM) to about micromolar (μM).

2. The method of Claim 1 wherein said compound is identified by recording the action potential duration (APD) of a sensory neuron as elicited by said compound relative to a control condition in a cell culture screening assay; selecting a compound which shortens said APD and does not prolong said APD relative to the APD from said control condition when said compound is assayed in the concentration range of about fM to about μM; and thereby identifying said low- or non-addictive analgesic.

3. The method of Claim 2 wherein said cell culture screening assay comprises exposing a dorsal-root ganglion (DRG) neuron to said compound, applying an intracellular depolarizing current to said DRG neuron, and recording said APD.

4. A non-addictive opioid analgesic capable of evoking an inhibitory effect on an opioid receptor-mediated function and which is not capable of evoking an excitatory effect on said function when said compound is present at a concentration ranging from about femtomolar fM to about μM.

5. The analgesic of Claim 4 wherein said analgesic is etorphine, dihydroetorphine, or ohmefentanyl.

6. A non-addictive opioid analgesic produced by the process of Claim 1.

7. A method of treating opioid addiction which comprises administering an effective amount of a non-addictive opioid analgesic of any one of Claims 4 to 6 to a patient for a first time sufficient to relieve or suppress

withdrawal symptoms of said opioid addiction, and subsequently administering decreasing amounts of said analgesic for a second time sufficient to wean said patient from said analgesic.

8. The method of Claim 7 wherein said analgesic is etorphine, dihydroetorphine, or ohmefentanyl.

9. The method of Claim 7 or 8 wherein said amount is from about 10 μg to about 1000 μg per day.

10. The method of Claim 7, 8 or 9 wherein said first time is from about 1 to about 5 days, said second time is from about 1 to about 7 days and the sum of said first time and said second time is from about 2 to about 12 days.

11. A method of treating opioid addiction which comprises administering about 40 to about 500 μg of dihydroetorphine to a patient for about one to about three days, administering decreasing amounts of dihydroetorphine for the following about 4 to about 7 days and providing no further dihydroetorphine by about 10 days after first administering said dihydroetorphine.

12. A method of treating opioid addiction which comprises administering an effective amount of a non-addictive opioid analgesic, to a patient for a first time sufficient for immediate relief or suppression of withdrawal symptoms due to said opioid addiction; administering an effective amount of an replacement opioid for a second time sufficient to maintain said relief or said suppression; followed by administering decreasing amounts of said non-addictive opioid analgesic for a third time sufficient to wean said patient from said analgesic.

13. The method of Claim 12 wherein said first administration of said non-addictive opioid analgesic and said administration of said replacement opioid is made simultaneously.

14. The method of Claim 12 or 13 wherein said analgesic is etorphine, dihydroetorphine, or ohmefentanyl.

15. The method of Claim 14 wherein said amount of said

analgesic is from about 10 μg to about 1000 μg per day.

16. The method of Claim 12 or 13 wherein said replacement opioid is methadone.

17. The method of Claim 16 wherein said amount of said replacement opioid is from 5 to about 100 mg/day.

18. The method of any one of Claims 12 to 17 wherein said first time is from about 1 to about 3 days, said second time is from about 1 to about 3 days, said third time is from about 1 to about 8 days and the sum of said first, second and third times is from about 3 to about 14 days.

19. The method of Claim 13 wherein said first and second times are a single time period of from about 2 to about 6 days and the sum of said period and said third time is from about 3 to about 14 days.

20. The method of any one of Claims 12 to 19 wherein any of said administerings of said non-addictive opioid or said replacement opioid is sublingually, intramuscularly or intravenously.

21. A method of treating acute or chronic pain which comprises administering an effective amount of dihydroetorphine for a time sufficient to relieve or suppress said pain without addiction or with low addiction, wherein said amount is about 10 μg to about 1000 μg.

22. The method of claim 21 wherein said amount for acute pain is about 20 to about 60 μg administered sublingually every three to four hours.

23. The method of Claim 21 wherein said amount for chronic pain is about 20 to about 100 μg administered sublingually every three to four hours.

24. The method of Claim 21 wherein said amount for acute pain is about 10 to about 30 μg administered intramuscularly every three to four hours.

25. The method of Claim 21 wherein said amount for chronic pain is about 10 to about 50 μg administered intramuscularly every three to four hours.

26. A method of treating acute or chronic pain which

comprises co-administering an effective amount of a low- or non-addictive opioid analgesic and an effective amount of a replacement opioid for a time sufficient to relieve, suppress or alleviate said pain without addiction or with low addiction.

27. The method of Claim 26 wherein said analgesic is dihydroetorphine, etorphine, ohmefentanyl or a pharmaceutically-acceptable salt thereof.

28. The method of Claim 26 wherein said amount of said analgesic is about 10 μg to about 1000 μg.

29. The method of claim 27 wherein said analgesic is dihydroetorphine and said amount of said analgesic for acute pain is about 20 to about 60 μg administered sublingually.

30. The method of Claim 27 wherein said analgesic is dihydroetorphine and said amount of said analgesic for chronic pain is about 20 to about 100 μg administered sublingually.

31. The method of Claim 27 wherein said analgesic is dihydroetorphine and said amount of said analgesic for acute pain is about 10 to about 30 μg administered intramuscularly.

32. The method of Claim 27 wherein said analgesic is dihydroetorphine and said amount of said analgesic for chronic pain is about 10 to about 50 μg administered intramuscularly.

33. The method of Claim 26 wherein said replacement opioid is morphine, methadone or fentanyl.

34. The method of Claim 33 wherein said amount of said replacement opioid is about 5 mg to about 100 mg per day.

35. The method of Claim 26 wherein said replacement opioid is morphine and said analgesic is dihydroetorphine or a pharmaceutically acceptable salt thereof.

36. A pharmaceutical composition comprising a low- or non-addictive opioid or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of Claim 36 further comprising a replacement opioid.

38. The pharmaceutical composition of Claim 36 further comprising naloxone.

39. The composition of any one of Claims 36 to 38 wherein said non-addictive opioid is dihydroetorphine, etorphine, ohmefentanyl or an analogue thereof.

40. The composition of anyone of Claims 36 to 39 wherein said non-addictive opioid is dihydroetorphine hydrochloride.

41. The pharmaceutical composition of Claim 40 wherein said composition is in a dosage form for a sublingual tablet comprising about 20 μg to about 40 μg of said dihydroetorphine hydrochloride.

42. The pharmaceutical composition of Claim 40 where said composition is in an injectable dosage form containing about 20 μg to about 100 μg of said dihydroetorphine hydrochloride.

43. The pharmaceutical composition of Claim 37 or 38 wherein said amount of said analgesic is from about 10 μg to about 1000 μg per day.

44. The pharmaceutical composition of Claim 37 wherein said replacement opioid is methadone, morphine, fentanyl or buprenorphine.

45. The pharmaceutical composition of Claim 37 or 44, wherein said amount of said replacement opioid is from about 5 mg to about 100 mg per day.

46. The pharmaceutical composition of Claim 37 wherein said analgesic is dihydroetorphine, or a pharmaceutically-acceptable salt thereof, and said replacement opioid is methadone.

47. The pharmaceutical composition of Claim 37 wherein said analgesic is dihydroetorphine, or a pharmaceutically-acceptable salt thereof, and said replacement opioid is morphine.

48. A method for preparing dihydroetorphine and

analogues thereof which comprises reacting thebaine with an excess of methyl vinyl ketone for a time and under conditions sufficient to produce a first product and recovering said first product; subjecting said first product to catalytic hydrogenation to produce a second product and recovering said second product; reacting said second product with a Grignard reagent of the formula RMgX for a time and under conditions to produce a third product and recovering said third product; reacting said third product with a strong base in an anhydrous solution for a time and under conditions sufficient to produce said dihydroetorphine or said analogue thereof; wherein R is a lower alkyl group and X is a halogen.

49. The method of Claim 48 wherein R is n-propyl or iso-amyl.

50. The method of Claim 48 which comprises reacting said dihydroetorphine or said analogue with an acid to form the corresponding salt and recovering said salt.

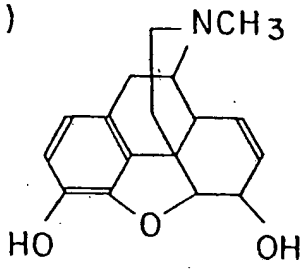
51. A method of preparing etorphine or an analogue thereof which comprises reacting an excess of methyl vinyl ketone with thebaine for a time and under conditions to produce a first product and recovering said first product; reacting said first product with a Grignard reagent of the formula RMgX for a time and under conditions to produce a second product and recovering said second product; reacting said second product with a strong base in an anhydrous solution for a time and under condition to produce said etorphine or said analogue thereof; wherein R is lower alkyl and X is a halogen group.

52. The method of Claim 51 wherein R is n-propyl, n-butyl, n-amyl, iso-amyl or cyclohexyl.

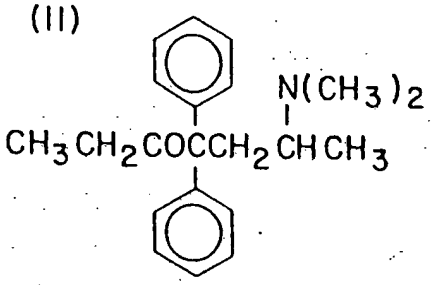
53. The method of Claim 51 which comprises reacting said etorphine or said analogue with an acid to form the corresponding salt and recovering said salt.

FIG. 1

(I)

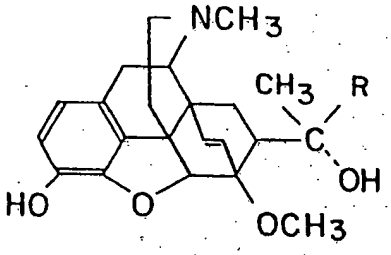



(II)



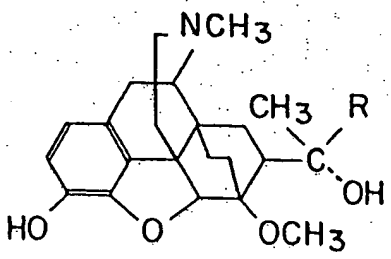

(III)

(a) R:n-propyl
(b) R:n-butyl
(c) R:n-amyl
(d) R:i-amyl
(e) R:cyclohexy




(IV)

(a) R:n-propyl
(b) R:i- amyl




(V)

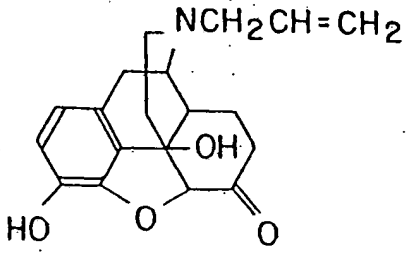


FIG. 2

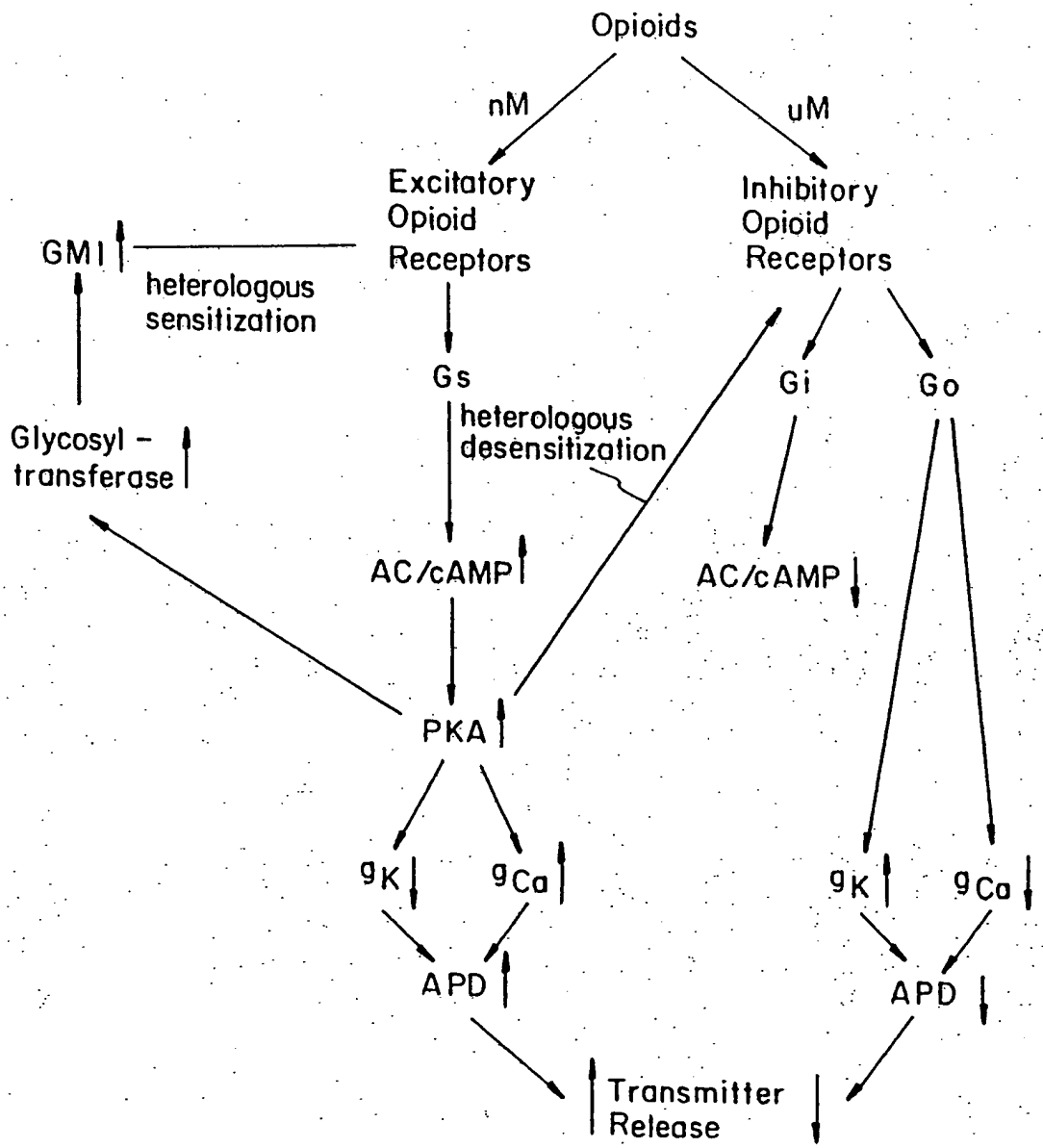
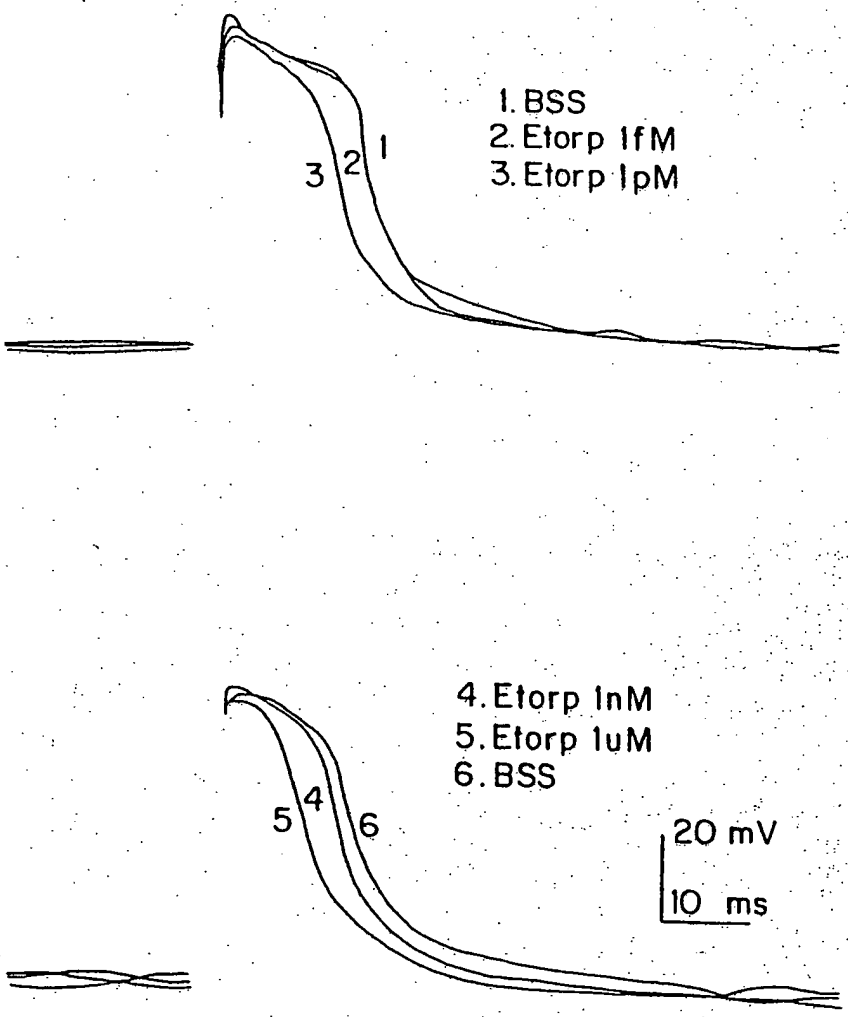

Opioids
nM
uM
Excitatory Opioid Receptors
Inhibitory Opioid Receptors
GM1
heterologous sensitization
Gs
Gi
Go
heterologous desensitization
Glycosyl – transferase
AC/cAMP
AC/cAMP
PKA
$g_K$
$g_{Ca}$
$g_K$
$g_{Ca}$
APD
APD
Transmitter Release

FIG.3


1. BSS
2. Etorp 1fM
3. Etorp 1pM
4. Etorp 1nM
5. Etorp 1uM
6. BSS
1
2
3
4
5
6
20 mV
10 ms

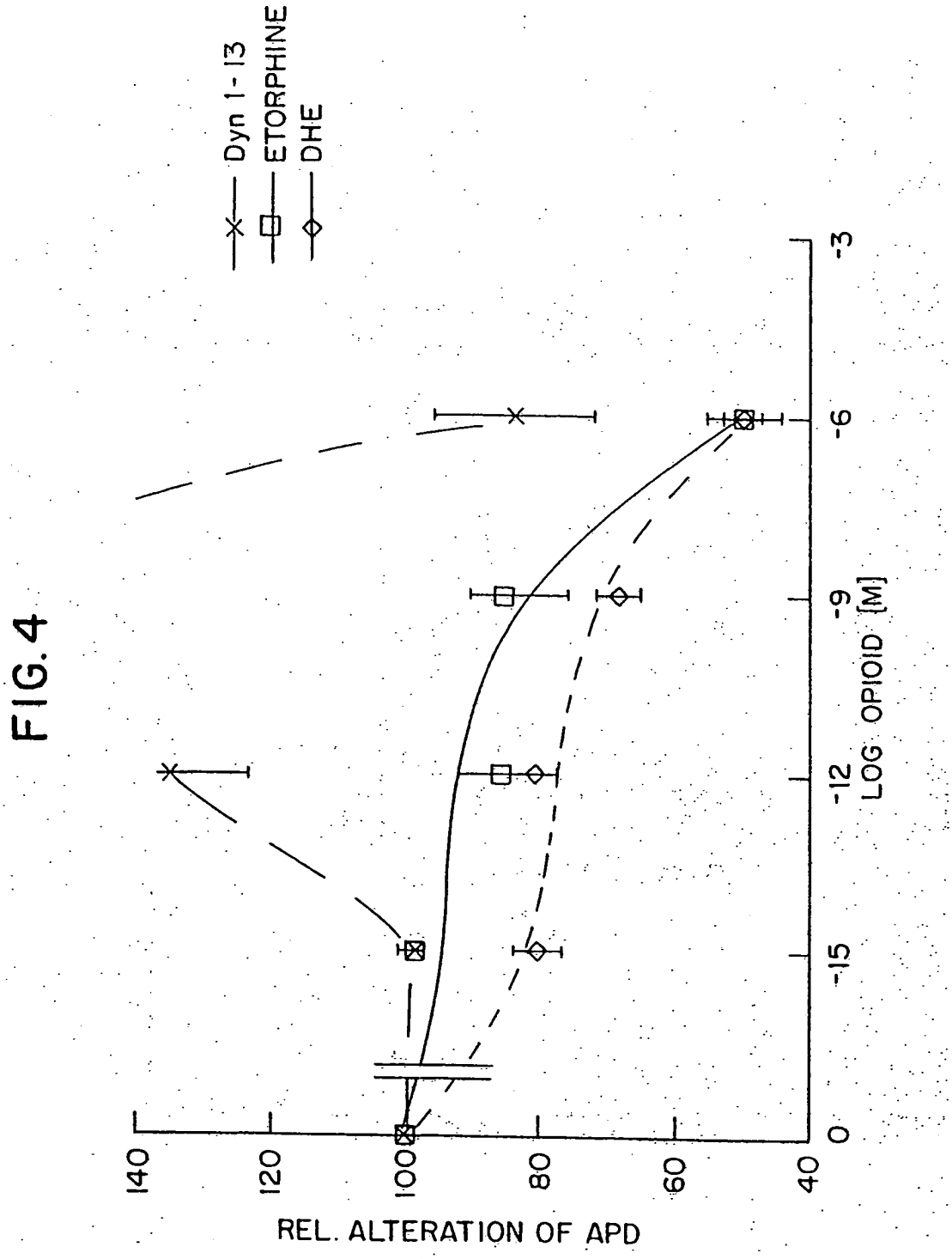

Dyn 1-13
ETORPHINE
DHE
-3
-6
-9
-12
-15
0
LOG OPIOID [M]
140
120
100
80
60
40
REL. ALTERATION OF APD

FIG. 4

FIG. 5

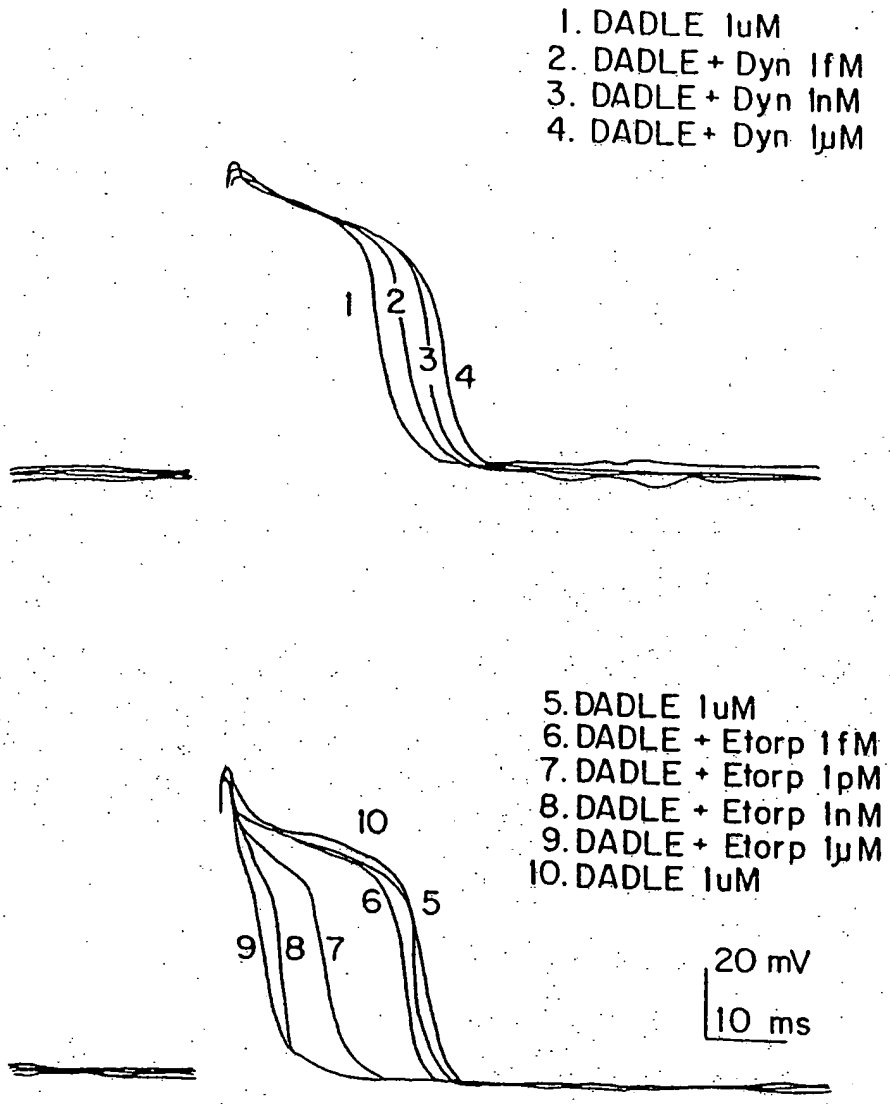

1. DADLE 1uM
2. DADLE + Dyn 1fM
3. DADLE + Dyn 1nM
4. DADLE + Dyn 1μM
1
2
3
4
5. DADLE 1uM
6. DADLE + Etorp 1fM
7. DADLE + Etorp 1pM
8. DADLE + Etorp 1nM
9. DADLE + Etorp 1μM
10. DADLE 1uM
10
6
5
9
8
7
20 mV
10 ms

FIG.6

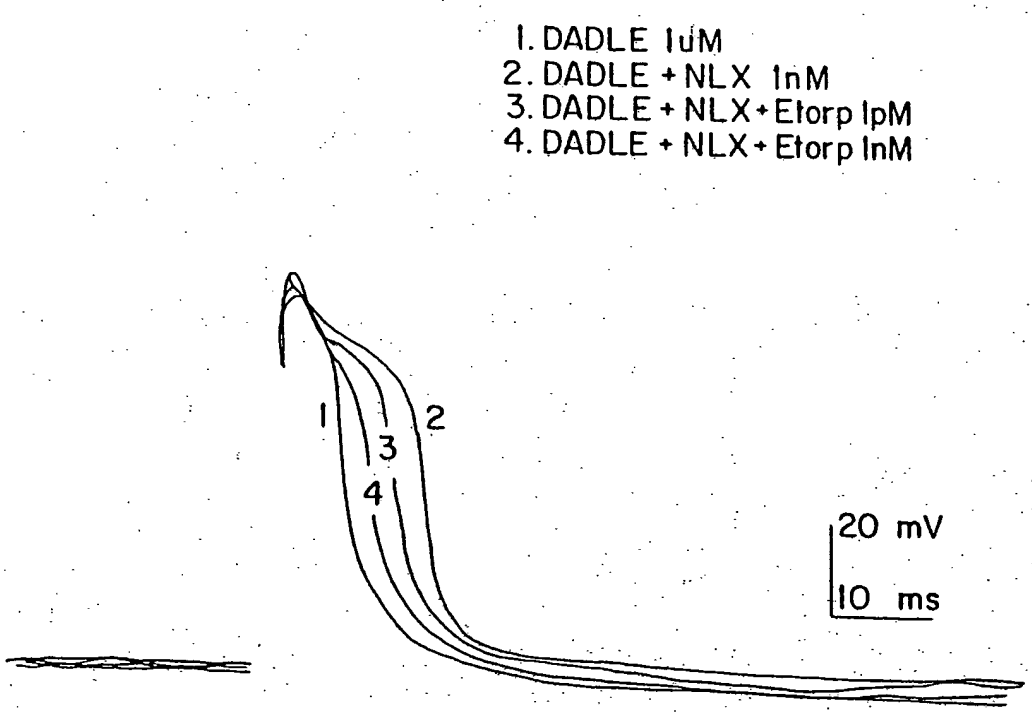

1. DADLE 1uM
2. DADLE + NLX 1nM
3. DADLE + NLX + Etorp 1pM
4. DADLE + NLX + Etorp 1nM
1
2
3
4
20 mV
10 ms

FIG. 7

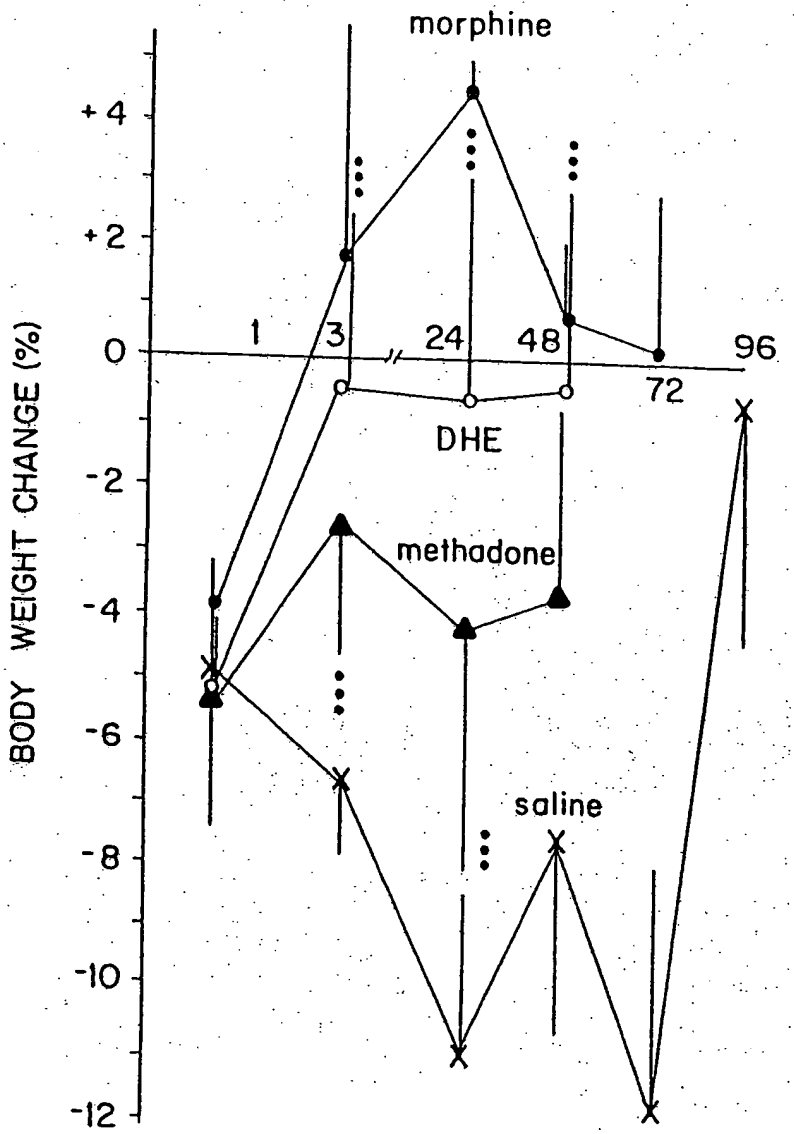

morphine
+4
+2
0
-2
-4
-6
-8
-10
-12
BODY WEIGHT CHANGE (%)
1
3
24
48
72
96
DHE
methadone
saline

FIG. 8

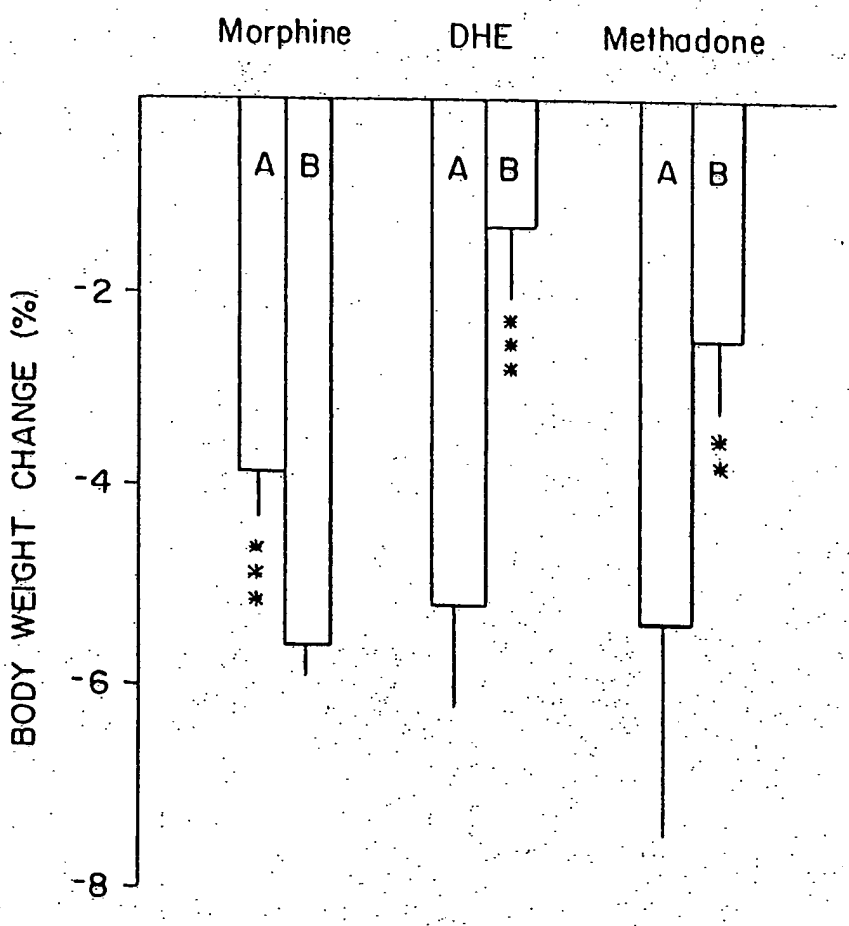


FIG. 9

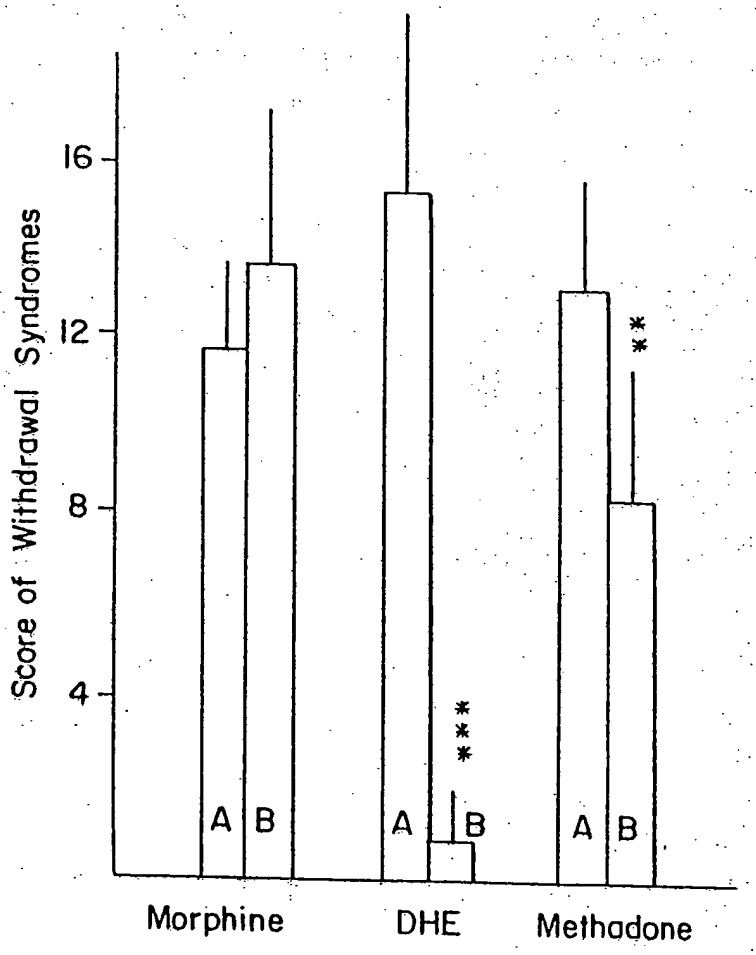

Score of Withdrawal Syndromes
16
12
8
4
A
B
A
B
A
B
***
**
Morphine
DHE
Methadone

FIG. 10

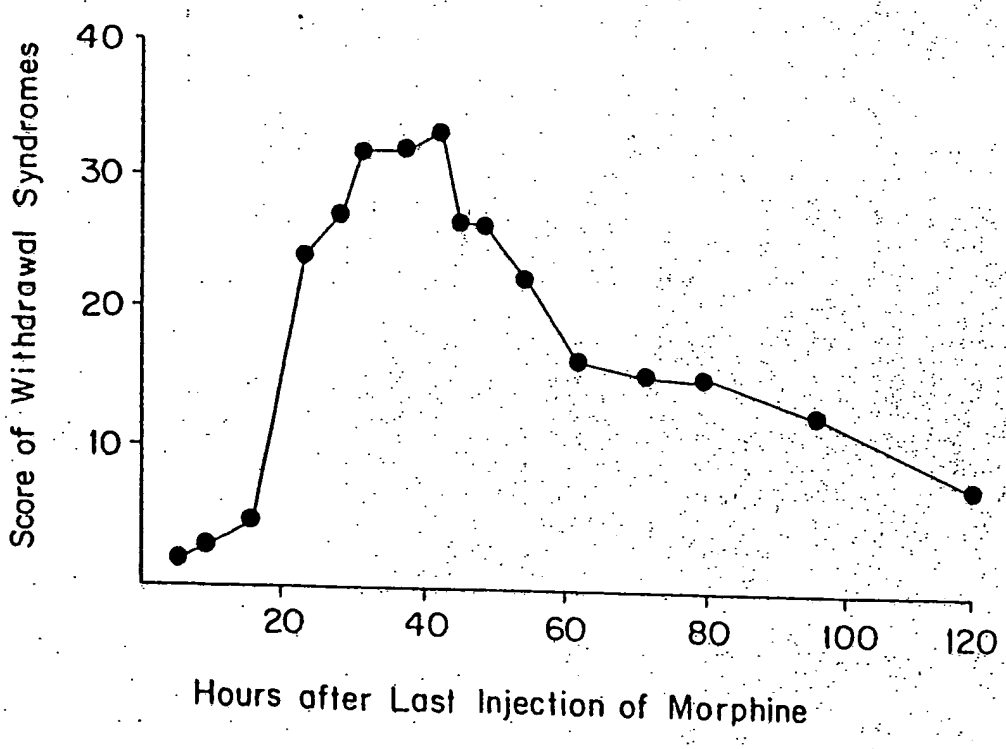

Score of Withdrawal Syndromes
40
30
20
10
20
40
60
80
100
120
Hours after Last Injection of Morphine

FIG. 11

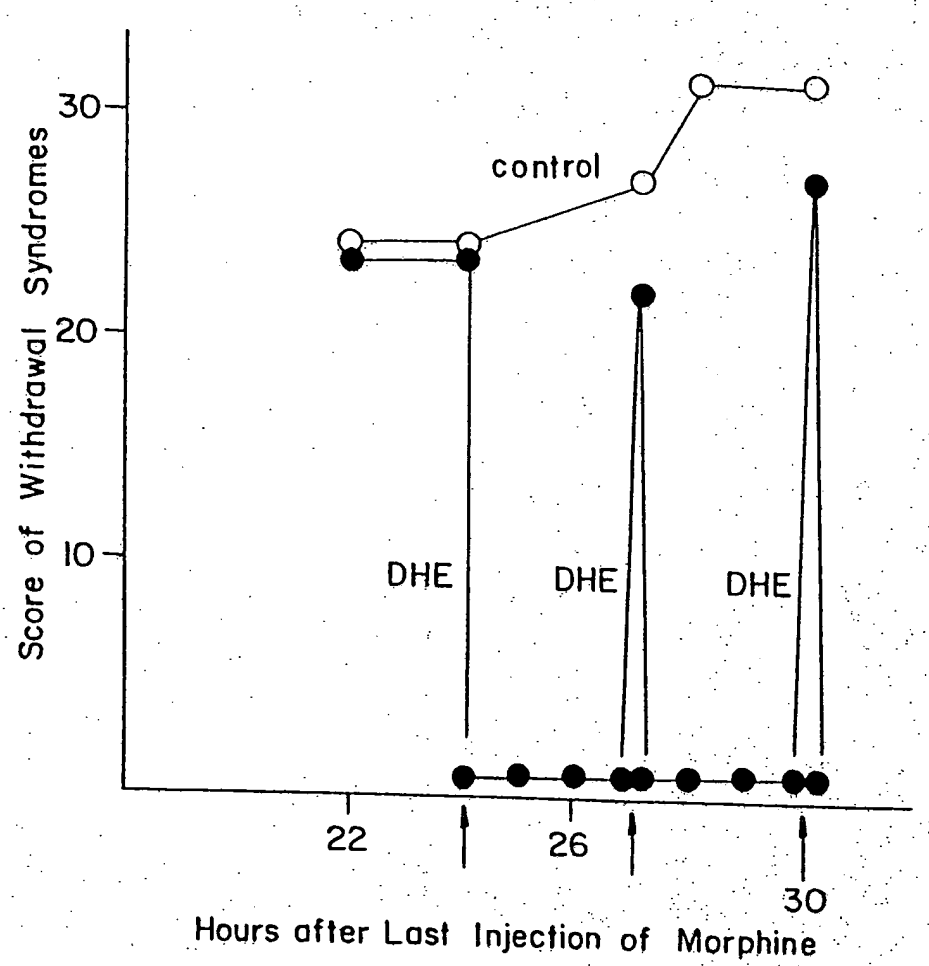

Score of Withdrawal Syndromes
30
20
10
control
DHE
DHE
DHE
22
26
30
Hours after Last Injection of Morphine

FIG. 12

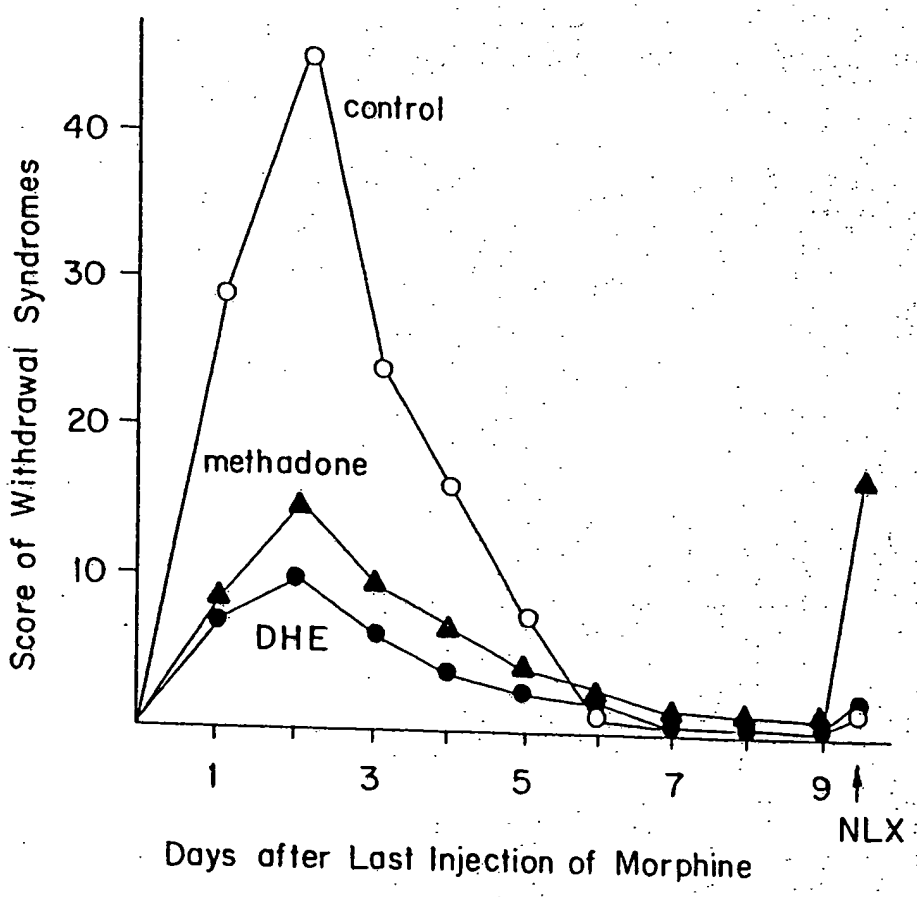

control
methadone
DHE
Score of Withdrawal Syndromes
40
30
20
10
1
3
5
7
9
NLX
Days after Last Injection of Morphine

FIG. 13

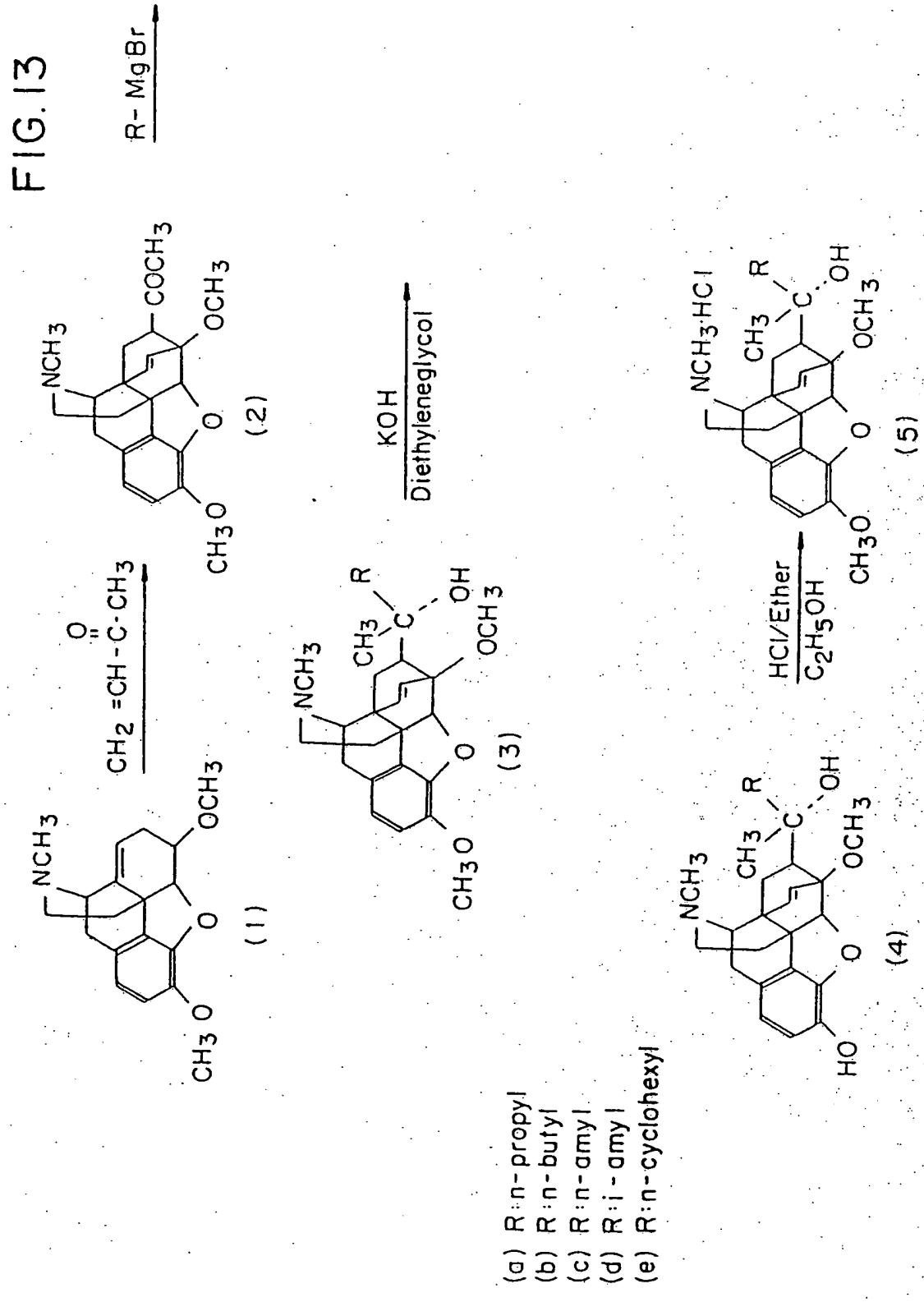

R-MgBr
KOH
Diethyleneglycol
HCl/Ether
$C_2H_5OH$
$CH_2=CH-\overset{O}{\overset{\|}{C}}-CH_3$
(1)
(2)
(3)
(4)
(5)
(a) R:n-propyl
(b) R:n-butyl
(c) R:n-amyl
(d) R:i-amyl
(e) R:n-cyclohexyl

FIG. 14

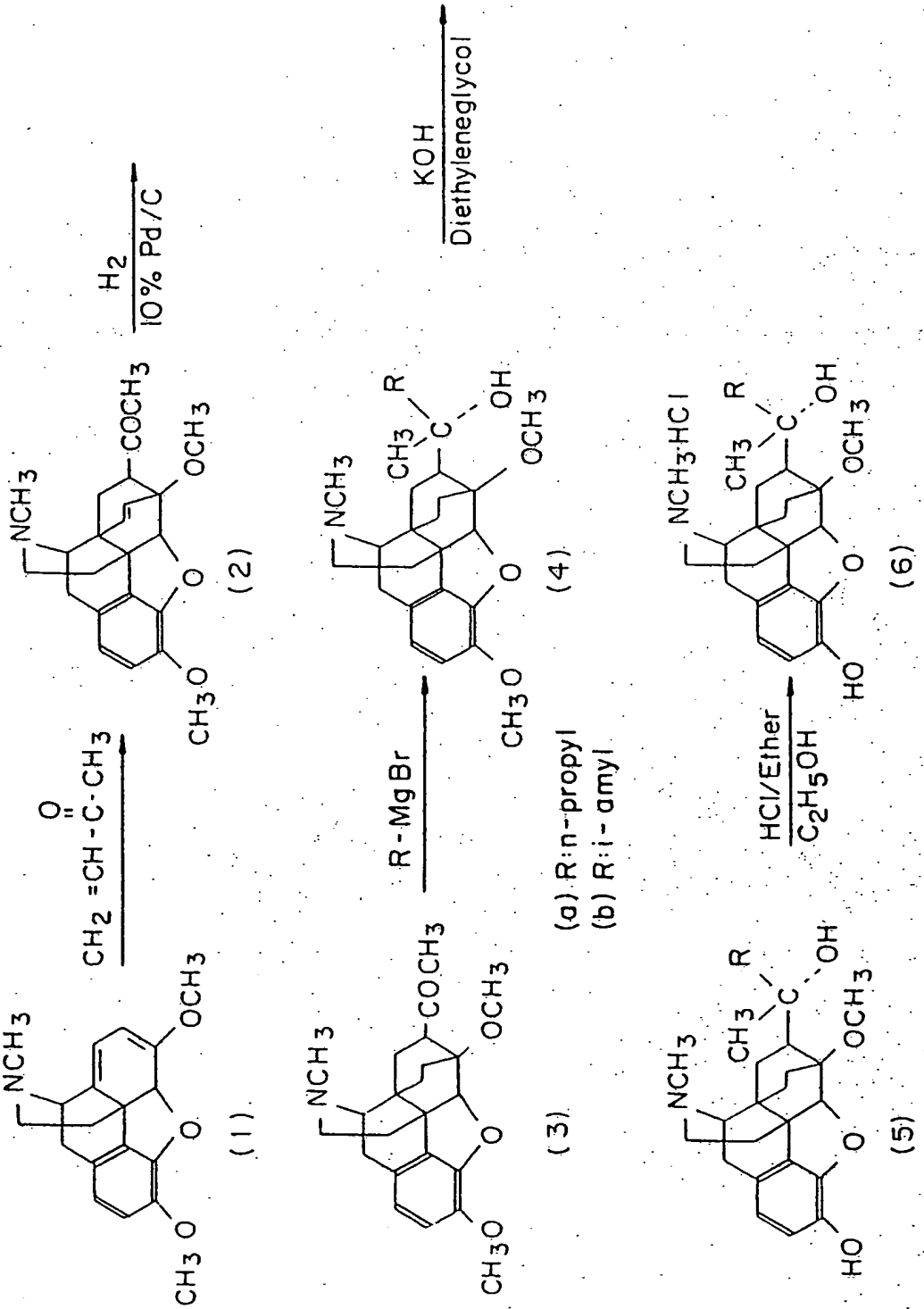
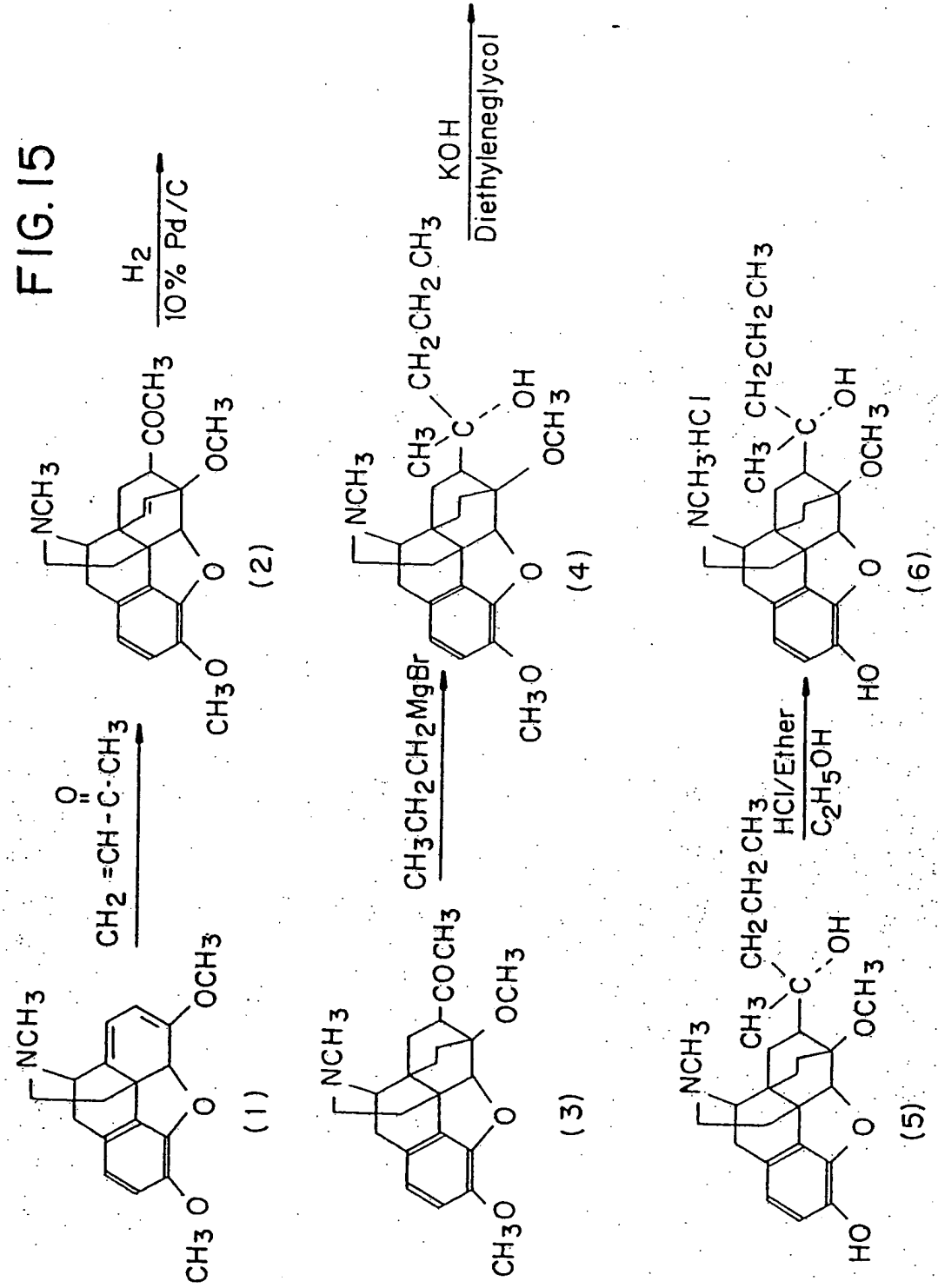

CH2 =CH-C-CH3
O
H2
10% Pd/C
KOH
Diethyleneglycol
R-Mg Br
HCl/Ether
C2H5OH
NCH3
COCH3
OCH3
CH3O
(1)
(2)
(3)
(4)
(5)
(6)
(a) R:n-propyl
(b) R:i-amyl

FIG. 15


NCH3
COCH3
OCH3
O
CH3O
(1)
(2)
(3)
(4)
(5)
(6)
CH2 =CH-C-CH3
O
H2
10% Pd/C
CH3CH2CH2MgBr
KOH
Diethyleneglycol
HCl/Ether
C2H5OH
CH3
CH2CH2CH3
OH
HO
NCH3·HCl

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08869

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/46; C07D 489/12; G01N 33/567

US CL :514/279; 546/39; 435/7.21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/279; 546/39; 435/7.21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS

search terms: etorphine, dihydroetorphine, ohmefentanyl, neuron

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | BEHAVIORAL NEUROSCIENCE, Volume 97, Number 5, issued October 1983, B. Thorn-Gray et al., "Rat brain sites responsive to etorphine: analgesia and catatonia", pages 768-778, see page 769, first paragraph and page 771 "Discussion". | 4-6<br>----<br>21-36 |
| Y | PROC. SOC. EXP. BIOL. MED., Volume 131, Number 1, issued 1969, M. Williams et al., "Analgesic tolerance to etorphine (M99) and morphine in the mouse", pages 97-100, see page 97, first paragraph and page 99, "Summary and Conclusion". | 7-20 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 02 December 1993 | 05 JAN 1994 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>MARY E. CEPERLEY<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08869

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | J. PHARM. PHARMAC., Volume 19, issued 1967, G. Blane, "Absence of respiratory depression in the new-born rat after maternal administration of etorphine by the sublingual route", pages 781-782, see page 781, first paragraph. | 4-6<br>----<br>21-36 |
| A | J. BIOL. CHEM., Volume 265, Number 1, issued 05 January 1989, B. Attali et al., "Opiate agonists inhibit Ca 2+ influx in rat spinal cord-dorsal root ganglion cocultures", pages 347-353, see abstract. | 1-3 |
| A | PROC. NATL. ACAD. SCI. USA, Volume 80, issued September 1983, T. Cho et al., "Isolation of opiate binding components by affinity chromatography and reconstitution of binding activities", pages 5178-5180, see page 5179, last paragraph. | 1-3 |
| A | BRAIN RESEARCH, Volume 517, Number 1-2, issued 1990, B. Attali et al., "Characterization of kappa opiate receptors in rat spinal cord-dorsal root ganglion cocultures and their regulation by chronic opiate treatment", pages 182-188, see page 182, last paragraph. | 1-3 |
| X | GB, A, 2,188,843 (SUGDEN) 14 October 1987, see claim 1. | 4-6, 21-36 |
| A | SCIENCE, Volume 199, issued 31 March 1978, R. MacDonald et al., "Specific-opiate-induced depression of transmitter release from dorsal root ganglion cells in culture", pages 1449-1451, see abstract. | 7-20 |
| A | US, A, 3,763,167 (HYDRO) 02 October 1973, see Example 1. | 48-53 |
| A | US, A, 4,906,655 (HORWELL ET AL.) 06 March 1990, see column 1, lines 14-24. | 7-20 |
| A,P | US, A, 5,192507 (TAYLOR ET AL.) 09 March 1993, see column 14, line 39 through column 15, line 9. | 1-3 |
| X | US, A, 4,829,056 (SUGDEN) 09 May 1989, see claim 1. | 4-6, 21-36 |
| X | US, A, 4,891,377 (SHIPMAN JR., ET AL.) 02 January 1990, see claims. | 4-6, 21-36 |